FORMULATION AND EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE CIPROFLOXACIN HCl MICROPARTICLES

A Dissertation submitted to
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI
In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

(PHARMACEUTICS)

Submitted by

Reg. No: 26104211

Under the guidance of
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MAY 2012

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled, **"FORMULATION AND EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE CIPROFLOXACIN HCl MICROPARTICLES"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, was carried out by **(Reg.No.26104211)** in the Department of Pharmaceutics, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the award of degree of Master of Pharmacy in Pharmaceutics under my supervision.

This work is original and has not been submitted in part or full for another degree or diploma of this or any other university.

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DECLARATION

The work presented in this thesis entitled **"FORMULATION AND EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE CIPROFLOXACIN HCl MICROPARTICLES"** was carried out by me in the Department of Pharmaceutics, Nandha College of Pharmacy, Erode-52 under the direct supervision of Assist. Prof. **P.Amsa, M.Pharm.,** Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other University.

Place: Erode **Reg. No. 26104211**

Date:

ACKNOWLEDGEMENT

First of all, I think the **god** who is constantly showering his blessing on me, it is my great privilege.

Pride and honour in expressing my humble thanks to my esteemed teacher and guide **Mrs. P.AMSA., M.Pharm, Asst. Professor**, Department of Pharamaceutics, Nandha College of Pharmacy, Erode for her valuable guidance, keen interest, inspiration and constant encouragement throughout the course of this investigation. Without her valuable advice and deep-rooted knowledge, this work would not have been a reality.

It is proud to express my sincere thanks to **Dr. T.Sivakumar, M.Pharm, Ph.D.**, principal of Nandha College of Pharmacy, with a deep sense of gratitude for his encouragement co-operation, kind suggestion and providing the best facilities during this work.

I express my profound indulge to **Dr. S.Tamilzharsi, M.Pharm, Ph.D, HOD., DEPARTMENT OF PHARMACEUTICS**, Nandha College of Pharmacy, Erode -52, for providing much of his effort in the form of suggestion, guidance, encouragement throughout the course of this thesis.

I express my loyal thanks to **Thiru.V. Shanmugam, B.Com, Chairman and Mr. Nandha Kumar Pradeep, M.B.A, Secretary**, Nandha College of Pharmacy, Erode-52, for providing all the facilities to make this work a success.

I owe my warm and humble thanks to **Mr. M.Jagadeeswaran, M.Pharm.,** Asst. Prof. Dept. of Pharm. Analysis, **Mr. K.Raja, M.Parm (Ph.D).,** Asst. Prof. Dept. of Pharmaceutics, **Dr.Sengottuvelu., M.Pharm.**, **Ph.D,** Departmet of Pharmacology, **Dr. R.DuraiSamy, M.Pharm.Ph.D.,** HOD, Dept. of Pharmacognosy. **Dr.P.R. Radhika, M.Pharm., Ph.D.,** Prof. Dept. of Pharmaceutics, kind suggestion and providing the best facilities during this work.

I express my deepest and special thanks to my batch mates **Mr. Ravi, Mr. Praveen, Mr.Sanath, Mr.Rajanish Reddy, Mr.Subhash, Mr.Prakash, Ms.Pavani, Mrs.Reepa Patel** for their kind of co-operation help and encouragement throughout my post- graduation.

I would like to thank for their immense help in doing my project in **Nandha College of Pharmacy.**

I also take opportunity to express my sincere thanks to all the teaching and non-teaching staff of Nandha College of Pharmacy for their kind co-operation and help throughout the course.

With no words I can hearties and deep gratitude to my dear friends who always believed In me and stood with me in good and bad times, special thanks to them for their friendship adherent love affection , encouragement they always showered on me. I am thanking to my juniors who have contributed directly and indirectly during my dissertation.

To completion of this dissertation is not only fulfillment of my dreams but also the dreams of my parents who have taken lots of pain for me in completion of higher studies.

A word thanks to all those gentle people associate with this work directly or indirectly whose names have been to unable to mention here.

Thank you to one and all……

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ABBREVATIONS

µg	-	Microgram
mg	-	Milligram
ng	-	Nanogram
gm	-	Gram
µm	-	Micrometer
nm	-	Nanometer
cm	-	Centimeter
ml	-	Mililitre
hrs	-	Hours
DDS	-	Drug Delivery System
CR	-	Controlled Release
SRM	-	Sustained Release Mucoadhesive
G.I.T.	-	Gastro Intestinal Tract
CMC	-	Carboxy Methyl Cellulose
HPMC	-	Hydroxy Propyl Methyl Cellulose
FN	-	Formulation Number
FT-IR	-	Fourier Transform Infra-Red

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1.0. INTRODUCTION

The drug should be delivered to site at the specific target sites at a rate and concentration that permit optimal therapeutic efficacy while reducing side effects to minimum. The other aspect to be considered in drug delivery is patient compliance during the drug therapy.

The concept of the advanced drug delivery systems especially those offering a sustained and controlled action of drug to desired area of effect, attained great appeal for nearly half a century. However, prior to advent of improved alternate methods, drug delivery systems were considered only as a means of getting the drug in to the patient's body. The actual practice of controlled release began with advent of timed release coating to the pills or solid drug particles in order to mask their unacceptable taste or make them more palatable.

In the mid 1940 - 1960s, the concept of chemical microencapsulation technology began as an alternative means of delivering drugs. In continued quest for the more refined systems, in 1980s polymer membrane technology came to be known at forefront. Further, the process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposomes, bio-erodible polymer, implants, monoclonal antibodies and various particulate carriers (e.g., nanoparticles and microspheres, etc.). The micro-particulate delivery systems are considered and accepted as a reliable means to deliver the drug to the target site with specificity, if modified and to maintain the desired concentration at the site of interest without untoward effect(s).

The term microcapsule, which explains as a spherical particle with size varying from 1µm to (1000 µm) 1 mm, containing a core substance. Microspheres comprises of strict sense, spherical empty particles. However, the terms microcapsules and microspheres are often used synonymously.

1.1. Microspheres[1, 2, 20]

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the drug to the target tissue in optimal amount at the right period of time thereby causing little toxic and minimal side effect[13]. There are various approaches in delivering a therapeutic substance to the site in a

sustained controlled release fashion. One such approach is using microspheres as carrier for drugs.

Definition of Microspheres:

The Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 μm^3.solid bio degradable microspheres incorporating a drug and dispersed or dissolved throughout particle matrix have the potential for the controlled release of drugs.

Microspheres are defined as homogeneous, monolithic particles in the size range of about 1µm-1000 µm and are widely used as drug carriers for controlled release. These systems have significant importance in biomedical applications. Microspheres can be produced for protection of core material, reduction of gastric irritation decrease in volatility, conversion of liquid to pseudo-solid, cell microencapsulation and for designing pulsatile drug delivery systems. Administration of the drug in the form of microspheres usually improves the treatment by providing the localization of the active substances at the site of action and by prolonging release of drugs.

Prerequisites for Ideal Carriers[3, 13]

- Longer duration of action
- Control of drug release
- Increased of therapeutic efficiency
- Protection of drugs
- Reduction of toxicity
- Biocompatibity
- Relative stability
- Water solubility or dispersability
- Targetability

1.2. Polymer used in Microspheres

These materials include the polymer of natural and synthetic origin and also modified natural substances

Synthetic Polymer

a. Non-Biodegradable

- Poly methyl methacrylate[9]
- Acrolein[11]
- Glycidyl methacrylate

b. Biodegradable

- Lactides and glycolides and their copolymers[25]
- Polyalkyl cyano acrylate

Natural Polymer

a. Proteins

- Albumins[29,31]
- Gelatin[30]
- Collagen

b. Carbohydrates

- Starch[24]
- Agarose
- Carrageenan
- Chitosan

c. Chemically modified carbohydrates

- Poly (acryl) dextran
- Poly (acryl) starch

In case of non-biodegradable drugs carriers, when administered parentrally the carrier remaining in the body after the drug is completely released poses possibity of carrier toxicity over a long period of time. Biodegradable carriers which degradable in the body to non-toxic degradation products do not pose the problem of carrier toxicity and are more suited for parenteral application[13].

1.3. Route of Administration

Microspheres can be used for the delivery of drugs via different routes. Route of administration is selected depending on the drug properties, disease state being treated and the age and condition of the patient. Desirable properties of the microspheres to be used for the delivery will also change depending on the route of administration.

1.3.1 Oral Delivery:

Oral delivery is the simplest way of drug administration. In oral drug delivery, the microspheres have to pass through frequently changing environments in the GI tract. There's also patient to patient variation in GI content, stomach emptying time and peristaltic activity. Although constraints of the oral route are numerous, on the whole, it offers less potential danger than the parenteral route. The relatively brief transit time of about 12 hrs through the GI tract limits the duration of action that can be expected via the oral route. Recently, it has been reported that microspheres of <10 μm in size are taken up by the payer's patches and may increase the retention time in the stomach. Also microspheres made from polymers with mucoadhesive properties get attached the stomach and prolong the residence time in the stomach. Bioavailability of the drugs with limited solubility in the stomach or intestine and small absorption rate constant can be increased by increasing the retention time in the stomach. improved drug delivery was observed compared to the microspheres administered alone.

1.3.2 Parenteral Delivery:

Most of the microsphere based controlled delivery systems are developed with the aim of using them for parenteral administration. Drug released is completely absorbed in this case. Microspheres used for parenteral delivery should be sterile and should be dispersible in suitable vehicle for injection. Hydrophilic microspheres have the potential advantage of aqueous dispersibility as opposed to hydrophobic microspheres for reconstituting them for injection. Surfactants in small concentrations are often necessary for reconstituting hydrophobic particles for injection in aqueous vehicles which are reported to cause adverse tissue reactions and affect the release of incorporated drug.

Drug Loading and Drug Release Kinetics [3, 13]

The active components are loaded over the microspheres principally using two methods, i.e. during the preparation of microspheres or after the formation of the microspheres by incubating them with the drug protein. The active components can be loaded by means of the physical entrapment, chemical linking and surface adsorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer.

Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives (e.g. cross linking agent,surfactant,stabilizers,etc.) heat of polymerization, agitation intensity etc. Release of the active constituents is an important consideration is case of microspheres.

Mechanism of Drug Release[1, 3, 13]

- Osmotically driven burst mechanism
- Pore diffusion mechanism
- Erosion or degradation of the polymer

a) Osmotically Driven Burst Mechanism:

Water diffuse into the core through biodegradable or non-biodegradable coating, creating sufficient pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecules/Polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres.

b) Pore Diffusion Method:

Penetrating water front continue to diffuse towards the core.

c) Erosion:

Loss of polymer is accompanied by accumulation of the surrounding release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix.

1.4. General Methods of Preparation[1, 3]

The Microsphere can be prepared by using any of the several techniques discussed in the following

- Single emulsion technique
- Double emulsion technique
- Polymerization technique
  - **a.** Normal polymerization
    - Bulk polymerization
    - Suspension polymerization
    - Emulsion polymerization
  - **b.** Interfacial polymerization
- Phase separation coacervation techniques
- Spray drying and spray congealing
- Solvent extraction
- Solvent evaporation technique

But the choice of technique mainly depend on the nature of polymer used, the drug, the intended use and the duration of therapy

Some formulation and technology related factors are mentioned below

- The particle size requirement
- The drug or the protein should not be adversely affected by the process
- Reproducibility of the release profile and method
- No stability problem
- There should be no toxic products associated with the final product.

Method of Preparation

Preparation of Microsphere should satisfy certain criteria:

- The ability to incorporate reasonably high concentration of the drug
- Stability of the preparation after synthesis with a clinically acceptable shelf life
- Controlled particle size and dispersability in aqueous vehicles for injection
- Release of active drug with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradability and
- Susceptibility to chemical modification.

Single Emulsion Technique:

The micro particulate carriers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymer are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like liquid paraffin.

Next cross linking of the dispersed globule is carried out. The cross linking is achieved either by means of heat or by using the chemical cross linkers. E.g. the chemical cross linking agents are glutaraldehyde, formaldehyde, Di acid chloride etc.

Disadvantages

- Heat denaturation is not suitable for thermolabile substance.
- Chemical cross linking suffers the excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing and separation.

Double Emulsion Technique:

Double emulsion method of involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited for water soluble drugs, peptides, proteins and vaccines.

This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase.

The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA).This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like LH-RH agonist, vaccines proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/extraction.

Polymerization Techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

a. Normal polymerization

b. Interfacial polymerization both are carried out in liquid phase.

a. Normal Polymerization

It is carried out using different technique as bulk, suspension, precipitation, Emulsion and micellar polymerization.

- **Bulk Polymerization Technique**

  Monomer or a mixture of monomer along with the initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization.

- **Suspension Polymerization Technique**

  Suspension polymerization also referred as bead or pearl polymerization is carried out by heating the monomer or mixture of monomer as droplets dispersion in a continuous aqueous phase. The droplet may also contain an initiator and other additives.

- **Emulsion Polymerization Technique**

  It is differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles.

b. Interfacial Polymerization

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to from a film of polymer that essentially envelopes the dispersed phase.

Phase Separation Coacervation Technique:

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates.

In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent result in the solidification of polymer.

Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of

achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particle.

The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetics of the formed particles since there is no defined state of equilibrium attainment.

Spray Drying and Spray Congealing

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively.

The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100µm.

Microparticles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying.

Advantage

Feasibility of operation under aseptic condition. The spray drying process is used to encapsulate various penicillin's. Thiamine mononitrate of mono and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous microparticles.

Solvent Extraction

Solvent extraction method is used for the preparation of microparticles, involves removal of the organic phase by extraction of organic solvents such as isopropanol .organic phase is removed by extraction with water. This process decrease the hardening time for the microspheres.

One variation of the process involves direct addition of drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the

temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer.

Solvent Evaporation[1, 4]

Solvent evaporation is carried out by maintain emulsion at reduced pressure or by stirring the emulsion so that the organic phase evaporation out. In the latter case, the emulsion is added to the large quantity of water into which organic phase diffuse out. The solid microspheres are subsequently obtained by filtration and washing.

Advantage

- High encapsulation efficiency of moderately water soluble and water-insoluble compounds
- Water soluble drugs such as theophylline, caffeine, and salicylic acid could be loaded efficiently using an o/o emulsion method.
- Versatile and easy
- It's possible to achieve various drug release profiles by the regulation of copolymer ratio, molecular weight, and size of the microsphere, drug loading, porosity and other formulation parameter.

1.5. Applications of Microspheres[1, 3, 13]:

Microspheres in Vaccine Delivery:

The prerequisite of a vaccine protection against the microorganism or its toxic product. An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. The aspect of safety and minimization of adverse reaction is a complex issue. The aspect of safety and the degree of the production of antibody responses are closely related to mode of application. Biodegradable delivery systems for vaccines that are given by parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages like:

- Improved antigenicity by adjuvant action
- Modulation of antigen release
- Stabilization of antigen

Targeting Using Micro particulate Carriers

The concept of targeting i.e. site specific drug delivery is a well-established dogma, which is gaining full attention. The therapeutic efficacy of the drug release on its access and specific interaction with its candidate receptors. The ability to leave the pool in reproducible, efficient and specific manner is center to drug action mediated by use of a carrier system. Placement of the particles indiscrete anatomical compartment leads to their retention either because of the physical properties of the environment or biophysical interaction of the particle with the cellular content of the target tissue.

Monoclonal Antibodies Mediated Microspheres Targeting

Monoclonal antibodies targeting microspheres are immuno microspheres. This targeting is a method used to achieve selective targeting to the specific sites. Monoclonal antibodies are extremely specific molecule. This extreme specificity of monoclonal antibodies (Mabs) can be utilized to target microspheres loaded bioactive molecule to selected sites. Mabs can be directly attached to the microspheres by means of covalent coupling. The free aldehyde groups, amino groups or hydroxyl groups on the surface of the microspheres can be linked to the antibodies. The Mabs can be attached to microspheres by any of the following methods

- Non specific adsorption
- Specific adsorption
- Direct coupling
- Coupling via reagent

Chemoembolization

Chemoembolization is an endovascular therapy, which involves the selective arterial embolization of a tumor together with simultaneous or subsequent local delivery the chemotherapeutic agent. The theoretical advantage is that such embolizations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of the tumor. Chemoembolization is an extension of traditional percutaneous embolization techniques.

Imaging

The microspheres have been extensively studied and used for the targeting purposes. Various cells, cell lines, tissue and organs can be imaged using radio labeled microspheres. The particle size range of Microspheres is an important factor in determining the imaging of particular sites. The particle injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is ex0ploited for the scintiographic imaging of the tumor masses in lungs using labeled human serum albumin microspheres.

Topical Porous Microspheres

Microsponges are porous microspheres having myriad of interconnected voids of particle size range 5-300 µm. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils etc., are used as the topical carriers system further, these porous microspheres with active ingredient can be incorporated into formulation such as creams, lotions and powders. Microsponges consist of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner.

Surface Modified Microspheres

Different approaches have been utilized to change the surface properties of carriers to product them against phagocytic clearance and to alter their body distribution patterns. The adsorptions of the poloxamer on the surface of the polystyrene, polyester or poly methyl methacrylate microspheres renders them more hydrophilic and hence decrease their MPS uptake. Protein microspheres covalently modified by PEG derivatives show decreased immunogenicity and clearance.

The most studied surface modifiers are:

- Antibodies and their fragments
- Proteins
- Mono, oligo and polysaccharides
- Chelating compounds (EDTA,DTPA or Desferroxamine)
- Synthetic soluble polymers

Such modification are provided surface of microspheres in order to achieve the targeting to the discrete organs and to avoid rapid clearance from the body.

1.6. Mucoadhesion[3, 4]:

Mucoadhesion is a promising approach in the design of the drug delivery systems to prolong the residence time of the dosage form the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs. Several studies reported mucoadhesive drug delivery systems in the form of tablet, films, patches and gels for oral, buccal, nasal, ocular, and topical routes.

Oral route is the most convenient route for the delivery of most of the drugs because of more flexibility in designing of dosage form and administration of the dosage form. The oral drug delivery systems (DDS) designing depends upon various parameters such as type of delivery system, type of disease treated, length of the therapy, patient, properties of the drug. The main drawback of the conventional dosage forms is frequent administration of dosage form and targeting of the drug is not possible. To overcome this drawback novel drug delivery systems are developed. These novel drug delivery systems are of two types viz. Controlled drug delivery systems (CDDS), targeted drug delivery systems. CDDS will release the drug in a controlled manner in the GIT and they are not specifically delivering the drug to GIT sites. But in case of targeted drug delivery systems the drug will be released to a particular site of the GIT, e.g. colon specific DDS.

Bio adhesive is the term that describes the adhesion of a polymer to a biological substrate. If the polymer is adhered to lining of mucosal surface then it is known as mucoadhesion. When the dosage form adheres to mucus membrane or biological membrane it results in immobilization of drug carrying particles at the mucosal surface. Mucoadhesive or bio adhesive controlled release systems improve the effectiveness of a drug by maintaining the drug concentration between the effective and toxic levels, inhibiting the dilution of the drug in the body fluids, and allowing targeting and localization of a drug at specific site. Bio adhesive systems can prevent first pass metabolism of certain protein drugs by liver through the introduction of drug via route bypassing the digestive tract.

1.7. Fundamentals of Bioadhesion

When the bio adhesive system come in contact with the biological membrane it forms an adhesive bond between the polymer and the biological membrane by forming contact between the two surfaces and forming secondary bonds by noncovalent interactions. The bond formation between polymer and membrane depends upon properties of polymer and biological membrane.

Bioadhesive Polymers:

The bio adhesive property of the polymer depends upon various properties of the polymer. The properties involved are, molecular weight of the polymer, chain length and cross-linking density of the polymer, charges and ionization, hydrophilic functional groups and degree of hydration.

Two classes of polymers appear to be of interest for bio adhesion: hydrophilic polymers and hydrogels. The large class of hydrophilic polymers, those containing carboxyl groups exhibits the best bio adhesive properties Bio adhesive polymers include sodium alginate, methylcellulose, carboxy methyl cellulose, hydroxy methyl cellulose, and cationic hydrogels such as chitosan. In general, hydrogels have most often been used for bio adhesive drug delivery because of the belief that polymer-mucin chain entanglement is an essential component in bio adhesive bond formation. However, other factors, such as surface energy, surface texture, electrical charge, and hydrophilic functional groups, may be equally important. It has recently been shown that nonhydrogel polymers that are high in hydrophilic functional groups can also produce intense bio adhesive interactions and can be utilized to improve bioavailability of orally administered compounds.

1.8. Mechanism of Bioadhesion[23]

The mechanisms responsible in the formation of bio adhesive bonds are not fully known, however most research has described bio adhesive bond formation as a three step process**.**

Step 1: Wetting and swelling of polymer

Step 2: Interpenetration between the polymer chains and the mucosal membrane

Step 3: Formation of chemical bonds between the entangled chains.

Wetting and Swelling of Polymer (Wetting Theory):

The wetting and swelling step occurs when the polymer spreads over the surface of the biological substrate or mucosal membrane in order to develop an intimate contact with the substrate. The ability of the adhesive to spread spontaneously on mucin influences development of intimate contact between the mucoadhesive and mucin and consequently influences the mucoadhesive strength. Bio adhesives are able to adhere to or bond with biological tissues by the help of the surface tension and forces that exist at the site of adsorption or contact. Swelling of polymers occurs because the components within the polymers have an affinity for water.

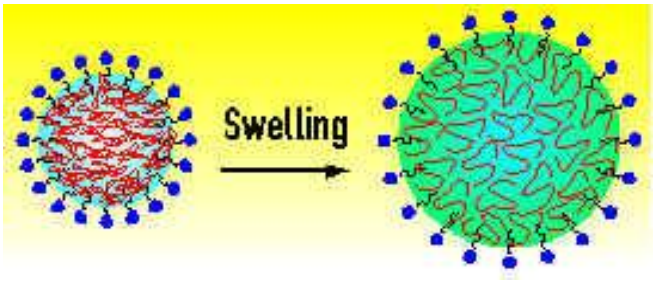


Fig: Swelling of a polymer

Interpenetration between the Polymer Chains and the Mucosal Membrane (Diffusion Theory):

The surfaces of mucosal membranes are composed of high molecular weight polymers known as glycoproteins. In step 2 of the bio adhesive bond formation, the bio adhesive polymer first brought into intimate contact with the mucous and over time the concentration gradient across interface causes the diffusion of the chains of the bio adhesive into mucous layer and also the diffusion of glycoprotein chains of the mucous into the bio adhesive polymer.

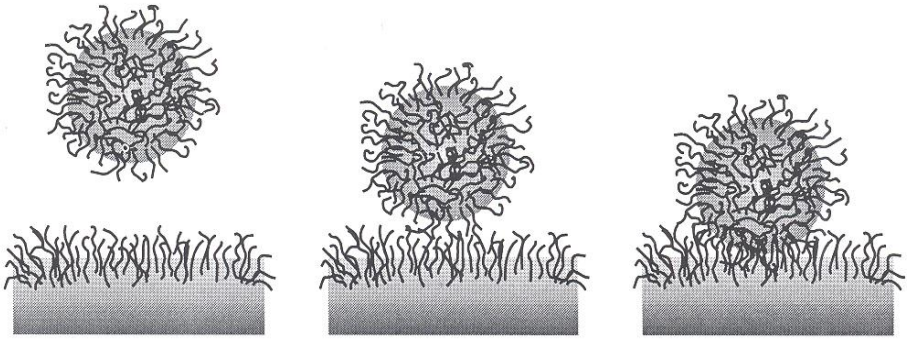


Fig: The interpenetration of polymer chains

Formation of Chemical Bonds between the Entangled Chains:

This step involves the formation of weak chemical bonds between the entangled polymer chains. The types of bonding formed between the chains include primary bonds such as covalent bonds and weaker secondary interactions such as Vander Waals Interactions and hydrogen bonds. Both primary and secondary bonds are exploited in the manufacture of bio adhesive formulations in which strong adhesions between polymers are formed.

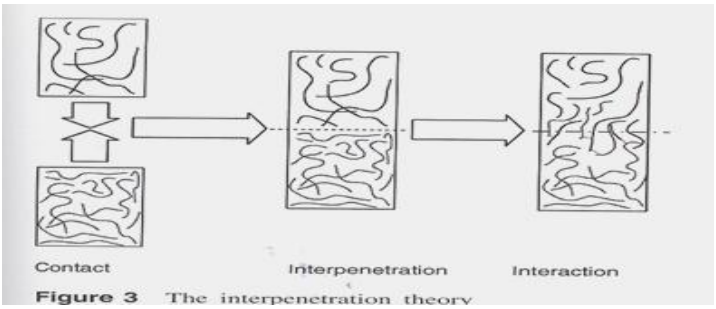


Figure 3 The interpenetration theory

Mechanisms of bio adhesion:

The formation of bond between bio adhesive polymer and mucous layer can also be explained by electronic theory. The mucin glycoprotein and mucoadhesive polymers possess different electronic structures; therefore electronic transfer is likely to occur when contact is made. This electron transfer results in formation of electric double layer at the adhesive interface with subsequent adhesion.

Factors affecting on Mucoadhesion

1. Polymer related factors:

i) Molecular weight

ii) Concentration of active polymer

iii) Flexibility of polymer chains

iv) Special confirmation

v) Swelling

2. Environment related factors:

i) pH of polymer - substrate interface

ii) Applied strength

iii) Initial contact time

3. Physiological factors:

i) Mucin turns over

ii) Disease state

Bioadhesion Advantages:

Advantages and applications of oral bio adhesive dosage forms are as follows:

a) Bio adhesive drug delivery systems can prolong GI transit time and improving oral drug absorption should ideally be nontoxic, non-absorbable from the GI tract.

b) They prolong the residence time at the site of action or absorption.

c) Preferably form a strong non-covalent bond with mucin-epithelial cell surfaces thereby they adhere to the tissues and cause localized drug release.

d) Adhere quickly to moist tissue.

e) Allow easy incorporation of drug and offer no hindrance to its release.

f) If pH sensitive polymers are used as bio adhesive material, the system will attach to pH specific sites and release the drug at those sites i.e. site specific drug delivery is

possible.

g) Economical:

Most of the routes of administration like ocular, nasal, rectal, buccal, respiratory, vaginal, are coated with the mucus layer, mucoadhesives increase the residence time and provide intimate contact between a dosage form and the absorbing tissue which results in high drug concentration in a local area and high drug flux through the absorbing tissue. The intimate contact may increase the total permeability of the high molecular weight drugs like proteins and peptides.

Microencapsulation:

It has been accepted as a process to achieve controlled release and drug targeting. Mucoadhesion has been a topic of interest in the design of drug delivery to prolong the residence time of dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs.

2.0. LITERATURE REVIEW

Alagusunduram. M *et al*.**, (2009)**[2] studied Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor. In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective *in-vivo* delivery and supplements as miniature versions of diseased organ and tissues in the body.

Meral Yuce *et al*.**, (2008)**[12] studied the Indomethacin loaded microspheres of ethyl cellulose were prepared by the emulsion solvent evaporation techniques. The aim of this work was to investigate the influence of process variation polymer type via viscosity grade of ethyl cellulose. Microspheres exhibited lower burst effect with decreased drug release rate, when the drug was incorporated with ethyl cellulose N100 and higher ratio of each polymer. Therefore Indomethacin release from ethyl cellulose microspheres could not be evaluated by any of the kinetic models.

Suja cjayan *et al*.**, (2009)**[14] studied about gelatin microspheres containing salbutamol sulphate prepared by coacervation phase separation method. And microspheres were characterized by optical microscopy and scanning electron microscopy. The microspheres were analyzed for drug entrapment and *in-vitro* release pattern. The batches of microspheres were prepared by altering drug polymer ratio and cross linking with glutaraldehyde. The size of microspheres was in the range of 5.6 m-22.4 mm the average diameter was found to be 12.34 mm. They were spherical in shape as evidenced by scanning microscopic photographs.

The percent drug entrapment was up to 80% and they could sustain drug release over a period of 8 and half hours

Malay and Das *et al.,* **(2006)**[15] designed diltiazem loaded mucoadhesive micro spheres by emulsification, internal gelatin techniques with a maximum incorporation efficiency of 93.29 ± 0.26% the scanning electron microscopy indicated that the microspheres were spherical in shape and drug remained dispersed in the polymer matrix. The *in-vitro* drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer. There was no significant change in drug content and cumulative drug release of drug loaded microspheres stored at different storage conditions after 8 weeks of study.

Rajendra Kotadiya *et al.,* **(2009)** [20] studied the theophylline loaded agar microspores. The effect of hydrocolloid on the physicochemical properties of the microspheres was investigated. The similar result of prepared formulation (t_{50}=211.78) and marketed product (t_{50}=209.29) suggest sustained release of the drug.

Rossoul dinarvand *et al.,* **(2005)**[21, 49] prepared gelatin micro spheres by polymerization techniques using glutaraldehyde as the cross linking agent. They investigated the effect of time of cross linking and amount of cross linking agent on the swelling properties of micro spheres and their release. The micro encapsulation efficiency micro spheres appearance, particle size, swelling ratio and drug release profile were also studied micro spheres with larger amount of cross linking agent showed reduced swelling ratio. *In-vitro* release pattern of lactic acid from gelatin micro spheres showed lymphatic profile and release rates were reduced upon increasing the amount of cross linking agent.

Shiv Shankar Hardenia *et al.,* **(2011)**[32] developed ethyl cellulose microspheres containing ciprofloxacin were prepared and evaluated for in-vitro performance of ciprofloxacin. Ciprofloxacin microspheres containing ethyl cellulose were prepared by emulsion solvent diffusion evaporation method. The surface morphological characteristics of ethyl cellulose microspheres were investigated using scanning electron microscopy. The polymer ratio, stirring speed and the temperature affected the particle size, shape and surface morphology of the microspheres. The in-vitro drug release was carried out using USP paddle type dissolution rate test apparatus in 0.1N HCL dissolution medium at 291 nm. It was found that drug release

from the formulations was different at different concentrations of polymers and different RPM and temperature. The best cumulative release was achieved after 24 hrs i.e. 91.6%. The Mucoadhesive property of the ethylcellulose microspheres was evaluated by in-vitro wash off test. The microspheres exhibited 75% mucoadhesion and showed good drug entrapment efficiency. By, above results it was concluded that ethyl cellulose microspheres showed reproducible results, with good Mucoadhesive properties and good surface morphology.

Bagherwal A *et al.***, (2010)**[41] Ciprofloxacin HCl belong to the fluoroquinoline derivatives which is widely used in the long term therapy for treatment of a wide range of infections including anthrax, biliary tract infection, bone and joint infection, gastrointestinal including traveler's diarrhea and *Campylobacter enteritis*, *Shiegella*, meningococcal meningitis prophylaxis, surgical infection prophylaxis, tuberculosis, leprosy and topically in the treatment of eye infections. Hence there is a potential need for floating tablet as sustained release dosage form for this drug. HPMC and carbomer are the polymers, used as suspending agent, viscosity increasing agent and tablet binder coating agents. In the present study, it was aimed to formulate floating tablet of ciprofloxacin HCl with HPMC and carbomer in different proportion (4%, 8% and 12%) by direct compression techniques using polymers lactose, Magnesium Streate, talc with sodium bicarbonate. All the prepared formulation were found to complies with the official tests like precompression parameter like angle of repose and post compression parameters like Shape, tablet dimensions, hardness, friability test, weight variation test, floating test, content uniformity and *in-vitro* dissolution study. *In-vitro* release studies were carried out using USP XXII dissolution test apparatus. The mean percentage of ciprofloxacin released at various time intervals was calculated and plotted against time. The mechanism of drug release with all the formulations was dominantly diffusion and followed zero order kinetics. It was observed that the integrity of the drug is not affected by formulation procedure. The results revealed the drug polymer ratio showed greater drug release than other formulations.

Hai H. Pham *et al.***, (2002)**[42] this investigation synthesized and characterized hydroxyapatite (HAP) microspheres, agglomerated microspheres, and implants containing ciprofloxacin. This delivery system is to be used as an implantable drug delivery system for the treatment of

bone infections. The HAP microspheres were made by chemical precipitation followed by a spray-drying technique. Agglomerated microspheres were prepared by a wet granulation process using a granulator. Implants were prepared by direct compression of the granules on a Carver press. Ciprofloxacin was analyzed by high-performance liquid chromatography. Characterizations of the HAP microspheres include particle size, size distribution, physical state of the drug in the microsphere, and microstructure of the drug delivery system before and after in vitro release. The particle size, porosity, and morphology of the microspheres were dependent on viscosity and concentration of the slurry as well as the atomization pressure used during spray drying. Even at the highest drug load (2% wt/wt), the drug was present in a noncrystalline state. The drug release from the agglomerated microspheres was quick and almost complete within 1 hour. However, Compressing the same amount of agglomerated microspheres into an implant greatly reduced the rate of ciprofloxacin release. Only 12% (wt/wt) of the drug was released from the implant within 1hr.

Jeong YI *et al***., (2009)** created ciprofloxacin HCl (CIP)-encapsulated poly (DL-lactide -co-glycoside) (PLGA) microspheres by the solvent evaporation method. Their antibacterial activity was evaluated with pathogenic microorganisms in vitro and in vivo. Since the half-life of CIP in the blood stream is short, sustained-release properties of microspheres may provide enhanced antibacterial activity. CIP- encapsulated microspheres of PLGA were prepared by the O/O method. CIP-encapsulated PLGA microspheres showed spherical shapes under a scanning electron microscope (SEM) and their particle sizes ranged from 10 to 50 micron. In an in vitro drug release study, CIP was continuously released over 3 weeks from the microspheres, and a burst effect was observed for the first 3 days. In the in vitro antibacterial activity test, CIP-microspheres showed lower antibacterial activity compared to free CIP because of their sustained-release properties, while empty microspheres did not affect the growth of microorganisms. In the in vivo antibacterial activity test, the number of microorganisms following treatment with CIP- encapsulated microspheres was significantly lower than after treatment with free CIP at 5 days post injection. These results suggest that encapsulated CIP keeps its antibacterial activity after microencapsulation.

Ravindra J. Salunke *et al***., (2009)**[43] the current study was to develop and optimize a Hydro dynamically balanced system of ciprofloxacin HCL as a single unit capsules using response

surface methodology. Ciprofloxacin HCl has an absorption window in the stomach and in the upper part of the small intestine. A 32 full factorial design was employed to optimize the formulation wherein hydroxylpropyl methyl cellulose K4M (HPMC K4M) (X1) and Carbopol 934 (X2) were taken as independent variables and amount of drug release after 12 hrs (Y1), t50 (Y2), and t85 (Y3) were taken as the dependent variables. The capsules were prepared by physical blending of drug and the polymers in varying ratios. The release data were evaluated by the model dependent (curve fitting) method using the PCP Disso v2.08 software. Optimization studies were carried out using the Design Expert Software (Version 7.1.6). Formulations were evaluated for *in vitro* buoyancy and *in vitro* release studies. The *in vitro* drug release followed zero order kinetics and the drug release mechanism was found to be anomalous or non-fickian type. It was found that both HPMC and Carbopol and their interaction had significant impact on the release and floating properties of the delivery system. The similarity factor *f*2 was found to be 62.32 for the developed formulation indicating the release was similar to that of the marketed formulation (Cifran). Thus, a combination of HPMC K4M and Carbopol 934 can be used to increase the gastric residence time and drug release for a period of 12hrs.

Dhakar R. C. ***et al*****., (2010)**[44] envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating Sustained Release Mucoadhesive (SRM) microspheres of Metformin hydrochloride (MH) for effective control of diabetes type-2. Microspheres were prepared by emulsification solvent evaporation method using Sodium carboxy methyl cellulose (SCMC), Carbopol 934P (CP) and Hydroxyl propyl methyl cellulose K4M (HPMC) as mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. The microspheres exhibits good mucoadhesive properties and showed high drug entrapment efficiency. MH release from these microspheres was slow and extended and dependent on the type of polymer used. The mean particle size decreased and the drug release rate increased at higher Stirring speed of emulsion content. Among all the formulations, formulation F1 containing SCMC and F2 containing CP showed the best reproducible results and mucoadhesive profile with good surface morphology. The data obtained thus suggest that mucoadhesive microspheres can successfully design for sustained delivery of MH and to improve patient compliance.

Faizi Muzaffar *et al*., **(2010)**[45] Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000μm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Amoxicillin microspheres were formulated by using solvent evaporation technique. Using Eudragit RS 100 as matrix polymer. Evaluation of the prepared mucoadhesive microspheres was done for % yield, particle size analysis, particle size distribution angle of repose, and determination of drug content, shape, surface characterization, drug entrapment and finally cumulative drug release from microspheres by suitable and reliable official methodologies.

Arunachalam.A *et al*., *(***2010)** in the present study, gelatin microspheres containing oflaxacin were prepared by coacervation phase separation method and characterized by optical microscopy and scanning electron microscopy. The microspheres were analyzed for drug entrapment, bulk density, angle of repose, particle size and *In-vitro* release pattern. The effect of process variables on microsphere size was studied and based on these preliminary studies, different batches of microspheres were prepared by altering the drug: polymer ratio and cross-linking with glutaraldehyde. The size of microspheres was in range of 42- 45 μm. They were spherical in shape as evidenced by photomicrographs and scanning electron microscopy. The percent drug entrapment was in the range of 78-90 % and they could sustain drug release over a period of 8 hrs.

Sindhuri.P *et al*., **(2011)**[46]Norfloxacin a fluoroquinoline derivative used as antibiotic requires multiple administration of drug, leading to fluctuation in plasma concentrations. The aim of present study is to formulate for sustained drug release. Norfloxacin microspheres (NM), by using various polymers like carbopol 934, Sodium Carboxy Methyl Cellulose (SCMC) using different drug: polymer ratios. Six formulations were prepared by using multiple emulsion solvent evaporation technique. NM were evaluated for parameters like angle of response, bulk density, particle size, drug content in microspheres, drug loading, encapsulation efficiency, In-vitro drug release studies. The prepared NM showed good flow properties, where spherical in shape with uniform surface morphology NM showed sustained release of the drug from the formulation for a period of 12 hours.

Pham H H *et al*.**, (2002**) synthesized and characterized hydroxyapatite (HAP) microspheres, agglomerated microspheres, and implants containing ciprofloxacin. This delivery system is to be used as an implantable drug delivery system for the treatment of bone infections. The HAP microspheres were made by chemical precipitation followed by a spray-drying technique. Agglomerated microspheres were prepared by a wet granulation process using a granulator. Implants were prepared by direct compression of the granules on a Carver press. Ciprofloxacin was analyzed by high-performance liquid chromatography. Characterizations of the HAP microspheres include particle size, size distribution, physical state of the drug in the microsphere, and microstructure of the drug delivery system before and after in vitro release. The particle size, porosity, and morphology of the microspheres were dependent on viscosity and concentration of the slurry as well as the atomization pressure used during spray drying. Even at the highest drug load (2% wt /wt), the drug was present in a noncrystalline state. The drug release from the agglomerated microspheres was quick and almost complete within 1 hour. However, compressing the same amount of agglomerated microspheres into an implant greatly reduced the rate of ciprofloxacin release. Only 12% (wt/wt) of the drug was released from the implant within 1 hour. The in vitro release of ciprofloxacin from these implants follows a diffusion-controlled mechanism. This method provides a unique way of producing various shapes and drug loads of HAP microspheres that can be easily manufactured on a commercial scale.

Venugopal Darak *et al*.**,(2011)**[47] A simple, Rapid and Reproducible HPLC method has been developed for the estimation of Mesalamine in bulk drug and its Pharmaceutical dosage forms using RP C18 column. The mobile phase consists of Acetonitrile and water in the ratio of 60:40 v/v and was pumped at a flow rate of 0.6 ml/min at 25±1^{o}c. The detection was carried out at 330 nm and the calibration curve was linear in the range of 20-100 µg/ml, Retention time was found to be 3.09 min for run time of 5 min. The method was statistically validated for its linearity, Precision and accuracy. Intra and Inter-day variation study was carried out and found to be less than 3% showing reasonable precision of the assay method. Parameters of validation obtained prove the accuracy of the method and its applicability for the determination of Mesalamine in tablet dosage formulations.

Duygu Gurcan *et al*.**, (2010)** study was to evaluate ciprofloxacin hydrochloride-loaded chitosan microspheres for nasal administration. Microspheres were prepared by spray drying method and evaluated with respect to the particle size, morphological properties, drug-polymer interaction, production yield, drug content, encapsulation efficiency, *in-vitro* drug release and kinetic assessment and *in-vivo* bioavailability. The particle size of microspheres prepared ranged from 3.3 to 6.7 μm. The microspheres showed spherical shape and smooth surface. For all formulations, drug loading capacity and microsphere yield were higher than 74% and 38%, respectively. Based on *in-vitro* evaluation of microspheres, the most suitable formulation has chosen for *in vivo* nasal application to rats. *In-vivo* studies showed that, absolute bioavailability of CIPRO formulations (oral solution, nasal solution and nasal microsphere suspension) were found as 8.57%, 15.7% and 32.9%, respectively. According to the obtained data, CIPRO-loaded chitosan microspheres prepared with spray-drying method are able to prove sustained release and could be use via nasal route as an alternative to oral route.

Tamizharasi S *et al*.**, (2009)**[48] prepared and evaluated poly (e-caprolactone) microspheres of Repaglinide by using the solvent evaporation techniques. The *in-vitro* release study showed that Repaglinide release from all formulations was slow and sustained over 12 hrs. Application of the in vitro drug release data to various kinetic equations indicated zero order release from Repaglinide microspheres.

Fatemeh atyali *et al*.**, (2004)**[49] prepared ethyl cellulose coated gelatin microspheres of 5-ASA. Gelatin and ethyl cellulose were used as primary and secondary polymer. Gelatin microspheres contain 5-amino salicylic acid was produced using solvent evaporation method. Gelatin microsphere showed no degradation in acidic medium. The gelatin microspheres were coated with ethyl cellulose by using co-acervation phase separation technique .The system provide suitable drug release pattern of active agent as 30% of the drug was released from the ethyl cellulose coated microcapsules within 6 hours while amount was 90% of the loaded drug for gelatin microspheres under the same condition.

3.0. DRUG AND POLYMER PROFILE

Drug Profile - CIPROFLOXACINE HCl[50]:

Ciprofloxacin HCl is a synthetic fluroquinone antibiotic.

Systematic (IUPAC) name: 1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl- quinolone- 3-Carboxylic acid.

Chemical data:

Molecular formula : $C_{17}H_{18}FN_3O_3$. HCl

Molecular weight : 331.346

Structure:

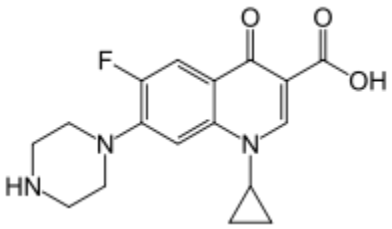


Fig: Structure of Ciprofloxacin

Physico-chemical properties:

Description : Yellowish to light yellowish crystalline powder.

Standards : Ciprofloxacin contains not less than 98.5 per cent and not more than of 101.5 %, calculated on the dried basis.

Solubility : Ciprofloxacin HCl is considered to be soluble in aqueous solutions with pH between 2 and 5. It is sparingly to slightly soluble in aqueous solutions with pH 7.4.

Melting point : Ciprofloxacin HCl melts at 255-257^0 C

Pharmacokinetic data:

Bioavailability : 69%

Metabolism : Hepatic, Including CYP1A2

Half-life : 4 hours

Extraction : Renal

Therapeutic considerations

Availability

Ciprofloxacin is available as:

- tablets (250 mg, 500 mg or 750 mg)
- intravenous solutions (5% and 10%, 100 ml)
- eye and ear drops
- In most countries, all formulations require a prescription.

Routes: Oral, intravenous, topical (ear drop & Eye drops)

Ciprofloxacin (INN) is a synthetic chemotherapeutic anti-biotic of the floroquinoline drug class. It is a second generation floroquinoline antibacterial. Kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops DNA and protein synthesis.

.STORAGE

- Store at room temperature below 86^0 F (30^o C) away from light and moisture. Do not store in the bathroom. Keep all medicines away from children and pets.
- Do not flush medications down the toilet or pour them into a drain unless instructed to do so. Properly discard this product when it is expired or no longer needed.
- Consult your pharmacist or local waste disposal company for more details about how to safely discard your product.

4.0. POLYMER PROFILE

Polymer: SODIUM CARBOXY METHYL CELLULOSE[33]

Grade : Pharma Grade (Standards of IP 96)

Prepared from cellulose by treatment with alkali and monochloro - acetic acid or its sodium salt. The article of commerce can be specified further by viscosity.

Synonyms : Sodium cellulose glycolate, Na CMC, CMC, cellulose gum, sodium CMC;

Chemical names: Sodium salt of carboxymethyl ether of cellulose

Chemical formula: [C6H7O2 (OH) x (OCH2COONa) y] n

Where,

n is the degree of polymerization

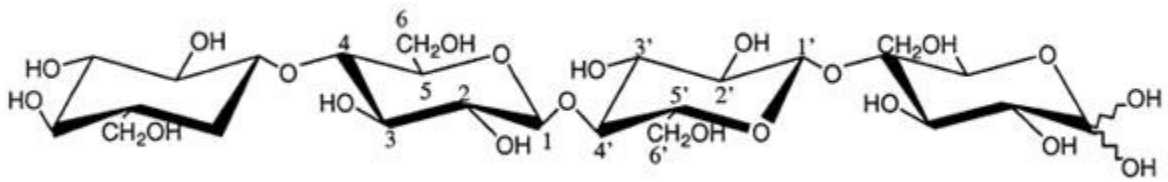
x = 1.50 to 2.80

y = 0.2 to 1.50

x + y = 3.0

(y = degree of substitution)

Structural formula:



Macromolecules: Greater than about 17,000 (n about 100)

Assay: Not less than 99.5 % of sodium carboxymethyl cellulose, calculated on the dried basis.

Description: White or slightly yellowish, almost odorless hygroscopic granules, powder

Or fine fibers.

Functional uses: Thickening agent, stabilizer, suspending agent characteristics.

Identification

Solubility: Yield viscous colloidal solution with water; insoluble in ethanol.

Foam test: Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxymethyl cellulose from other cellulose ethers and from alginates and natural gums.

Precipitate formation: To 5 ml of a 0.5% solutions of the sample add 5 ml of a 5% solution of copper sulfate or of aluminum sulfate. A precipitate appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers and from gelatin, carob bean gum and tragacanth gum).

Colour reaction: Add 0.5 gm of powdered carboxymethylcellulose sodium to 50 ml of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 ml of the solution, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube and carefully introduce down the side of the tube 2 ml of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

Purity: Loss on drying not more than 12% after drying (105^{o} C to constant weight)

pH: 6.0 - 8.5 (1 in 100 soln)

Sodium: Not more than 12.4 % on the dried basis. Determine total sodium content by Atomic Absorption Spectroscopy or Flame Photometry. Sodium chloride not more than 0.5% on the dried basis See description under tests.

Free glycolate: Not more than 0.4 % calculated as sodium glycolate on the dried basis

Degree of substitution: Not less than 0.20 and not more than 1.50.

Lead: Not more than 2 mg/ kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in "Instrumental Methods."

Properties Uses and Applications

Sodium Carboxy Methyl Cellulose is an anionic water soluble polymer derived from cellulose. It is odorless, tasteless and nontoxic. It has the following properties making it useful in a wide variety of applications.

1. Easy Solubility in cold and hot water.
2. Fine film forming properties.
3. Resistance to oil greases and solvents.
4. Better thickening action.
5. Physiological inertness
6. Anionic character
7. Binding properties
8. Suspending characteristics.
9. Gives transparent film.
10. Acts as a protective colloid reducing water losses.

Polymer: SODIUM ALGINATE

Nonproprietary Name:

- BP: Sodium Alginate
- Ph. Eur: Sodium Alginate
- USP-NF: Sodium Alginate

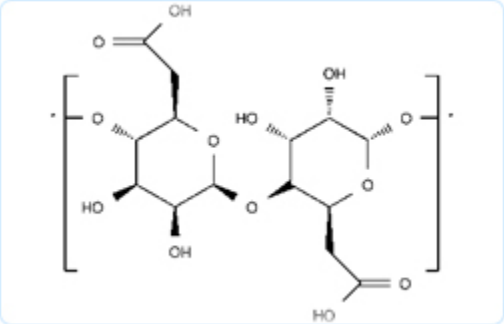
Synonyms: algin, alginic acid, sodium salt, sodium polymannuronate

Chemical Name: Sodium alginate

Empirical Formula:

Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic

Structure:



Molecular formula: $(C_6H_7O_6Na)$

Description:

White or light yellow, vagiform power, odorless, tasteless, dissolve in water, insoluble in ethanol and ether.

Method of Manufacture:

Alginic acid is extracted from brown seaweed and is neutralized with sodium bicarbonate to form sodium alginate.

Acidity/alkalinity: pH 7.2 for a 1% w/v aqueous solution.

Functional Category:

Stabilizing agent, suspending agent, tablet and capsule disintegrate, tablet binder, viscosity increasing agent.

Applications in Pharmaceutical Formulation or Technology:

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrate. It has been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions.

Description:

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

Solubility: Practically insoluble in ethanol (95%), ether, chloroform, and ethanol or water mixtures in which the ethanol content is greater than 30%. Slowly soluble in water, forming a viscous colloidal solution.

Viscosity (dynamic): Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 20^0C, will have a viscosity of 20–400 mPas (20–400 cP).

Stability and Storage Conditions:

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidity and a cool temperature.

Incompatibilities:

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%.

Method of Manufacture:

Alginic acid is extracted from brown seaweed and is neutralized with sodium bicarbonate to form sodium alginate.

Safety:

It is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may be harmful.

Applications:

- Sodium alginate is used in a variety of oral and topical pharmaceutical formulations.
- In tablet formulations, sodium alginate may be used as both a binder and disintegrates
- It has been used as a diluent in capsule formulations
- Sodium alginate has also been used in the preparation of sustained release oral formulations since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions.
- In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams and gels as a stabilizing agent for oil-in-water emulsions.

- Sodium alginate has been used for the aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic-solvent systems.
- It has also been used in the formation of nanoparticles.

Polymer: HYDROXY PROPYL METHYLCELLULOSE

1. Nonproprietary Name:

BP: Hypromellose

USP: Hypromellose

2. Synonyms:

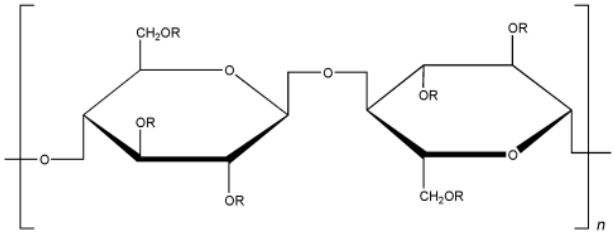
Hydroxypropyl methylcellulose, HPMC, hypromellosum, Methocel, methylcellulose propylene glycol ether, methyl hydroxypropylcellulose, Metolose, MHPC.

3. Chemical Name: Cellulose hydroxypropyl methyl ether.

4. Empirical Formula and Molecular Weight:

The PhEur 6.3 describes hypromellose as a partly O-methylated and O-(2-hydroxypropylated) cellulose. Molecular weight is approximately 10,000 – 1,500,000.

5. Structural Formula:



Structure of HPMC

Where R is H, CH_3, or CH_3CH (OH) CH2

6. Applications in Pharmaceutical Formulation or Technology:

Hypromellose is widely used in oral, ophthalmic, nasal and topical pharmaceutical formulations. In oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended release tablet formulations. Concentrations between 2% and 5% w/w may be used as a binder in either wet- or dry-granulation processes. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in

tablets and capsules. Hypromellose is also used in liquid oral dosage forms as a suspending and or thickening agent at concentrations ranging from 0.25 – 5.0%.

7. Description:

Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder.

8. Solubility:

Soluble in cold water, forming a viscous colloidal solution; practically insoluble in hot water, chloroform, ethanol (95%) and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane.

9. Viscosity (dynamic):

A wide range of viscosity types are commercially available. Aqueous solutions are most commonly prepared.

Typical viscosity values for 2% (w/v) aqueous solutions of methocel (Dow Chemical Co.) viscosities measured at 20ºC

Methocel grade	**Viscosity(cps)**
K4 M	4000
K15M	15000
K100M	100000

10. Stability and Storage Conditions:

Hypromellose powder should be stored in a well-closed container, in a cool, dry place.

11. Safety:

Hypromellose is generally regarded as a nontoxic and nonirritating material, although excessive oral consumption may have a laxative effect.

5.0. RESEARCH ENVISAGED

5.1. Aim of Work:

The objective of this study is to develop, characterize, and evaluate mucoadhesive microspheres of Ciprofloxacin employing various mucoadhesive polymers for prolonged gastrointestinal absorption.

Mucoadhesive are preferred because immobilization of drug carrying particles at the mucosal surface would result in:

- A prolonged residence time at the site of action or absorption.
- A localization of the drug delivery system at a given target site.
- An increase in drug – concentration gradient due to intestine contact of the particles with the mucosal surface.

A direct contact with intestinal cells, which is the step earlier to particle absorption.

5.2. Plan of Work:

The present work was carried out to prepare and evaluate microperticulate drug delivery system Ciprofloxacin HCl of using Sodium Carboxy Methyle Cellulose, Sodium Aliginate and Hydroxy Propyle Methyle Cellulose in various proportions. The following experimental protocol was therefore designed to allow a systemic approach to the study.

- Procurement of drug and raw materials.
- Pre- formulation studies for possible drug or polymer interaction by IR analysis and UV.
- Preparation of standard curves.
- Preparation of mucoadhesive microspheres by Emulsification Solvent Evaporation technique.
- Drug release study using suitable *in-vitro* model.
- Evaluation of the various properties of Mucoadhesive microspheres.
    a. Particle size analysis.
    b. Bulk density.
    c. Drug Entrapment efficiency.
    d. Drug Content.
    e. *In-vitro* mucoadhesion study
    f. Kinetic modeling.
    g. Scanning Elecron Microscopy
    h. HPLC
    i. Stability Studies

6.0. MATERIALS AND EQUIPMENTS:

MATERIALS:

The Materials used For the Process are as follows:

List of chemicals: Table: 1

S.No	Chemicals	Company
1.	Ciprofloxacin HCl	Darwin Formulations, Mumbai, India
2.	Sodium CMC	Reachem laboratory chemicals Pvt.Ltd, India
3	Sodium Aliginate	Rajesh Chemicals, Mumbai, India
4	HPMC	Dr.Reddy's Lab Pvt.Ltd, Hyderabad, India
5.	Seasem oil	AR grade, India
6.	Light Liquid paraffin	Qualigens fine chemicals, Mumbai, India
8.	Span 20	Sd fine –chem limited, Mumbai, India
9.	n-Hexane	Sd fine –chem limited, Mumbai, India

List of Equipment's: Table: 2

S.No	Equipment	Company
1.	Glass ware	DELTA (Borosilicate Glass)
2.	UV Spectrophotometer	Elico LI 120
3.	Mechanical stirrer	Remi motors
4.	USP type 11 Station Dissolution apparatus	ELECTRO Lab
5.	Balance	Dolphin Digital Balance
6.	FTIR	KBR press model M15
7.	HPLC	SPD-10 AVP, SHIMADZU

7.0. EXPEREMENTAL INVESTIGATION

Preformulation Study:

7.1. Organoleptic properties[34]:

Ciprofloxacin was observed for color, odor and taste.

7.2. Standard calibration curve of Ciprofloxacin HCl[34]:

Ciprofloxacin HCl can be estimated spectrometrically at 272 nm as it obeys Beer's – Lambert's law limit is the range of 2-20 µg/ml.

7.2.1. Preparation of 0.1 N Hydrochloric acid (pH 1.2):

8.5 ml of concentrated hydrochloric acid was taken and then diluted with distilled water up to 1000 ml (0.1N HCl).

A. Stock Solution

100 mg of Ciprofloxacin HCL was dissolved in 100 ml of 0.1N HCl. So, as to get a stock solution of 1000 µg/ml concentration.

B. Standard Solution

10 ml of stock solution was made to 100 ml with 0.1N HCl thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 0.2 ml to 1 ml were transferred in to 10 ml volumetric flask and were diluted up to the mark with 0.1N HCl. Thus the final concentration ranges from 2-10 µg/ml. Absorbance of each solution was measured at 272 nm against 0.1N HCl as a blank. A plot of concentrations of drug vs. absorbance was plotted.

7.3. Drug-excipients interaction study by FTIR Spectrum[45]:

FTIR study was carried out for Ciprofloxacin as well as for Excipients (Sodium CMC, Sodium Alginate, Hydroxy propyl Methyl Cellulose).

Emulsification Solvent evaporation technique

- Dissolve the coated substance in a volatile solvent (immiscible with liquid Manufacturing Vehicle, LMV)
- Dissolve or disperse the core material in coating polymer solution with agitation.
- Disperse the core coat mixture in LMV with continuous agitation.
- Heat the whole mixture to evaporate the solvent for the polymer.
- Cross linking with glutaraldehyde, formaldehyde etc.,

7.4. Emulsification Solvent Evaporation Method for Preparation of Mucoadhesive microspheres[46, 51]:

Mucoadhesive microspheres were prepared by the w/o emulsification by solvent evaporation technique. The drug was dissolved in each polymeric aqueous solutions. The solutions were poured into 200 ml of sesame oil containing span-20 (0.5%) as an emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in 500 ml beaker at 500 rpm by mechanical stirrer. The beaker and its contents were heated on the hot plate at 80˚C. Continues stirring and heating were maintained for 4 hrs until the aqueous phase was completely removed by evaporation. The light mineral oil was decanted and collected microspheres were washed three times with 100 ml aliquots of n-hexane, filtered through what man filter paper, dried in an oven at 50˚ C for 2 hrs and stored in a dessicator at room temperature. Microspheres were prepared using Sodium Alginate, Sodium Carboxyl Methyl Cellulose and HPMC polymers.

Composition of Mucoadhesive Microspheres of Ciprofloxacin HCl:

Table: 3

Sl. No	Formulations	Drug (mg)	Sodium CMC (mg)	Sodium Alginate (mg)	HPMC (mg)	Drug Ratio
1	F1	500	500	-----	-----	1:1
2	F2	500	1000	-----	-----	1:2
3	F3	500	-----	500	-----	1:1
4	F4	500	-----	1000	-----	1:2
5	F5	500	-----	-----	500	1:1
6	F6	500	-----	-----	1000	1:2

Formulation Procedure:

7.5. Evaluation of microspheres

7.5.1. Bulk density (D_b) [12, 15]:

It is the ratio of powder to bulk volume. The bulk density depends on particle size distribution, shape and cohesiveness of particles. Accurately weighed quantity of spheres was carefully poured into graduated measuring cylinder through large funnel and volume was measured which is called initial bulk volume. Bulk density is expressed in gm/cc and is given by,

$D_b = M / V_o$ Where, D_b = Bulk density (gm/cc)

M =Mass of powder (g)

V_o = Bulk volume of powder (cc)

Bulk density (gm/cm^3) = weight of sample / Final volume

7.5.2. Particle Size Analysis[18, 32]:

A minute quantity of microsphere was spread on a clean glass slide. This was placed in stage of calibrated optical microscope; approximately 100 microspheres were counted. The particle size was calculated in triplicate for each batch using the formula

Arithmetic mean = Σnd / sum of number of particle

Volume surface diameter = $\Sigma nd^3 / \Sigma nd^2$

7.5.3. Drug content[32]:

Ciprofloxacin content in the microspheres was estimated by a UV Spectrophotometric method based on the measurement of absorbance at 272 nm in distilled water. Microspheres equivalent to 50 mg were weighed and added in 100 ml of distilled water. The volumetric flask was stirred continuously for 24 hrs on a magnetic stirrer. Dilutions of the above solutions were made suitably and measured for the drug content.

7.5.4. Drug Loading and Entrapment Efficiency[13, 28,32]:

Ciprofloxacin microspheres were weighed and dissolved in 0.N HCl. The UV absorbance of the solution was measured using a (Shimadzu UV-100 serious, Japan) 272 nm. Drug loading and encapsulation efficiency were determined by following formula and the values were expressed as percentage.

$$\text{Drug Loading} = \frac{\text{weight of Ciprofloxacin}}{\text{weight microspheres}} \times 100$$

7.5.5. *In-vitro* wash-off test for mucoadhesion[32]:

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals at 37˚C, up to 3.5 hrs, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula.

% mucoadhesion = (no. of microspheres remains / no. of applied microspheres) ×100

7.5.6. *In-vitro* drug release study[42]:

Procedure:

In-vitro drug release studies of Ciprofloxacin HCl were conducted for a period of 12 hrs using USP XXIII type II apparatus at 37 $\pm$ 0.5^0 C and at 50 rpm speed in 900 ml of 0.1 N HCl (pH1.2). An aliquot of the sample was periodically with drawn at suitable time interval and volumes were replaced with fresh dissolution medium in order to maintain the sink condition. After withdrawing at predetermined time interval for 12 hrs, the samples were analyzed by a UV spectrophotometer (ElicoLI 120, Mumbai, India) at 272 nm. The study was performed in triplicate.

7.5.7. Kinetic Analysis of *In-Vitro* Release Rates of Ciprofloxacin HCl[37, 38]:

1. Zero order kinetics:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation –

$$Q_t = Q_o + K_o t$$

Where Q_t = amount of drug dissolved in time t, Q_o = initial amount of drug in the solution and K_o = zero order release constant.

2. First Order Kinetics:

First – order release would be predicted by the following equation:-

$$\text{Log } C = \log C_0 - K_t / 2.303$$

Where, C = Amount of drug remained at time‘t’

C_0 = Initial amount of drug.

K = First – order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant ‘K’ can be obtained by multiplying 2.303 with the slope values.

3. Higuchi model:

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. And the equation is

$$Q_t = K_H . t^{1/2}$$

Where Q_t = Amount of drug released in time t, K_H = Higuchi dissolution constant.

4. Krosmeyer and Peppas release model:

To study this model the release rate data are fitted to the following equation

$$M_t / M_\infty = K.t^n$$

Where M_t / M_∞ is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion.

Table 4: Mechanism of Drug Release as per Korsmeyer Equation / Peppa's Model

S. No.	N Value	Drug release
1.	0.45	Fickian release
2.	0.45 <n <0.89	Non – Fickian release
3.	n> 0.89	Class II transport

7.5.8. Morphology [32]:

The external and internal morphology of the Microspheres were analysed by scanning electron microscopy (SEM Hitachi S 502, Tokyo, Japan).

7.5.9. HPLC analysis of Ciprofloxacin HCl[42, 47]:

Drug:

The reference sample of Ciprofloxacin HCl was supplied by Darwin Formulations, Mumbai, India. The branded tablets formulation of Ciprofloxacin (Baycip) was purchased from local market.

Preparation of Mobile Phase:

Weigh **(**0.13 gm) potassium dihydrogenphaspate and dissolve in 100 ml of HPLC water to get the concentration of 0.01 M for the analysis the mobile phase consists of acetonitrile and 0.01 M potassium dihydrogenphaspatein the ratio of 70:30 v/v and adjusted the pH in to 4.5 by using glacial acidic acid and flow rate of 1 ml/min.

Preparation of Standard Solution:

Primary stock solution of ciprofloxacin prepared by dissolving 100 mg in 100 ml of mobile phase to get the concentration of 1 mg/ml. Further dilute 10 ml of this to 100 ml with mobile phase to get 100 mg/ml of ciprofloxacin HCl respectively. Ciprofloxacin shows maximum absorbance in UV at 272 nm.

Preparation of sample solution (Assay of Ciprofloxacin HCl in Microspheres):

Weighed 50 mg of ciprofloxacin and dissolved in 50 ml of mobile phase to get 1000 µg/ ml. Further dilute with 10 ml with mobile phase to get the concentration of 100 µgm/ml. The mixture was filtered through whattmann's filter paper No: 40 at each step and then sonicated it for 15 min. observe the absorption at the detector wave length of 272 nm.

Chromatographic Conditions:

The HPLC system consisted of a pump (VL cipro casting) programmed by a system controller (model Spinchran), an ultraviolet-visible spectrophotometric detector (LCATVP), and a recorder (model CR501) from Shimadzu (Tokyo, Japan). The separation was carried out using a phenomnx (C180 pH stable column (Phase Separations, Hyd), 15 cm long. The mobile phase consisted of glacial acetic buffer: acetonitrile: methanol (85:10:5 vole/vole/vole) with an apparent pH adjusted to 4.5 with Glacial acetic acid. The flow rate was maintained at 1.5 mL.min-1 and the column effluent was monitored at 272 nm.

Stationary Phase	Phenomnx C_{18} Analytical column (250×4.6 mm, 5 µm)
Mobile Phase	ACN: 0.01 M Potassium dihydrogen phosphate (70:30 v/v) and maintain the pH up to 4.5 by using glacial acetic acid.
Flow rate	1 ml/min
Dection wave length	272 nm
Injection volume	20 µl
Temperature	Ambient temperature
Run Time	15 min

Table 5: Chromatographic Conditions

7.5.10. Stability Study[39]:

Samples from each batch were withdrawn after the definite time intervals and the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test.

All the batches of Ciprofloxacin microparticles were tested for stability. The preparations were divided into 3 sets and were stored at 5-8^0 C (refrigerator) 27^0 C and at 40^0 C. After 15, 30 and 60 days drug content of all the formulations was determined by the method discussed previously in entrapment efficiency section.

8.0. RESULT AND DISCUSSION

The aim of the study was to design and evaluation of SRM microspheres of ciprpfloxacin HCl were prepared by emulsification solvent evaporation technique using span-20(0.5%) in seasem oil incorporating mucoadhesive polymers like sodium carboxy methyl cellulose, sodium alginate and HPMC with various ratios. Finally to carry out the stability studies for the selected formulations. The microspheres were prepared by using generally approved excipients that were compatible with the ciprofloxacin a fluoroquinolone analog.

Preformulation Parameters:

Organoleptic properties:

Ciprofloxacin was observed for color, odor and taste.

Determination of λmax of Ciprofloxacine HCl :

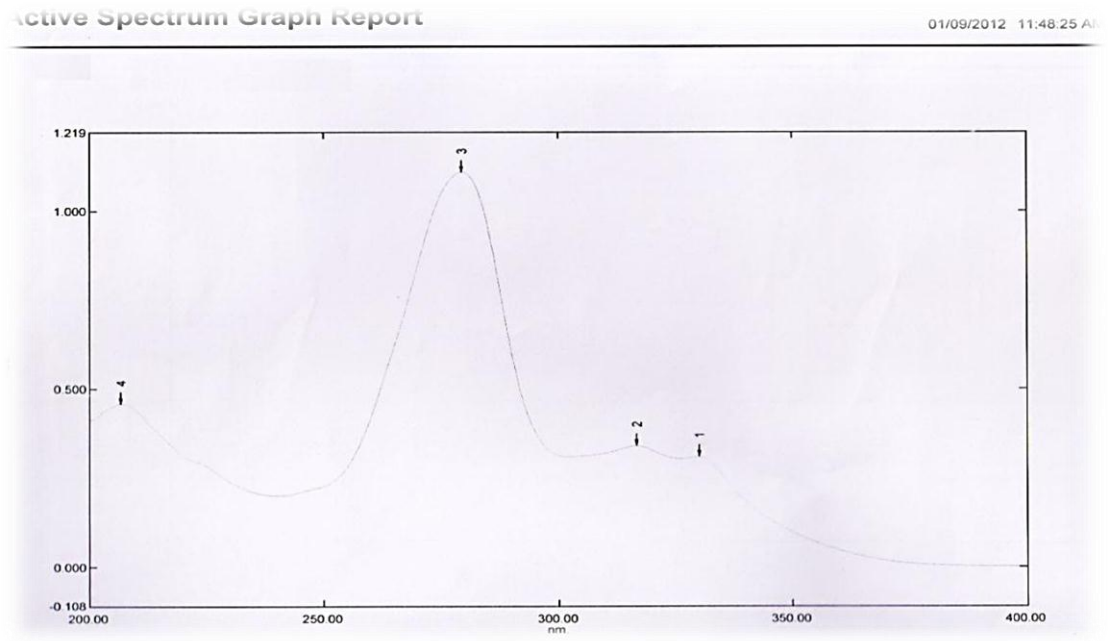


Fig 1: Spectrum of Ciprofloxacine HCl

Ciprofloxacine HCl drug solution in pH 1.2 (0.1N HCl) was scanned using UV-Spectrophotometer between the range 210-400 nm using pH 1.2 as blank and the maximum absorbance (λ max) was found at 272nm**.** It was concluded that the drug has λmax of 272 nm, which is exactly 272 nm as reported.

Calibration curve of Ciprofloxacin HCl:

Table 6:

S.No	Concentration(µg/ml)	Absorbance
1	0	0
2	2	0.161
3	4	0.376
4	6	0.552
5	8	0.743
6	10	0.901

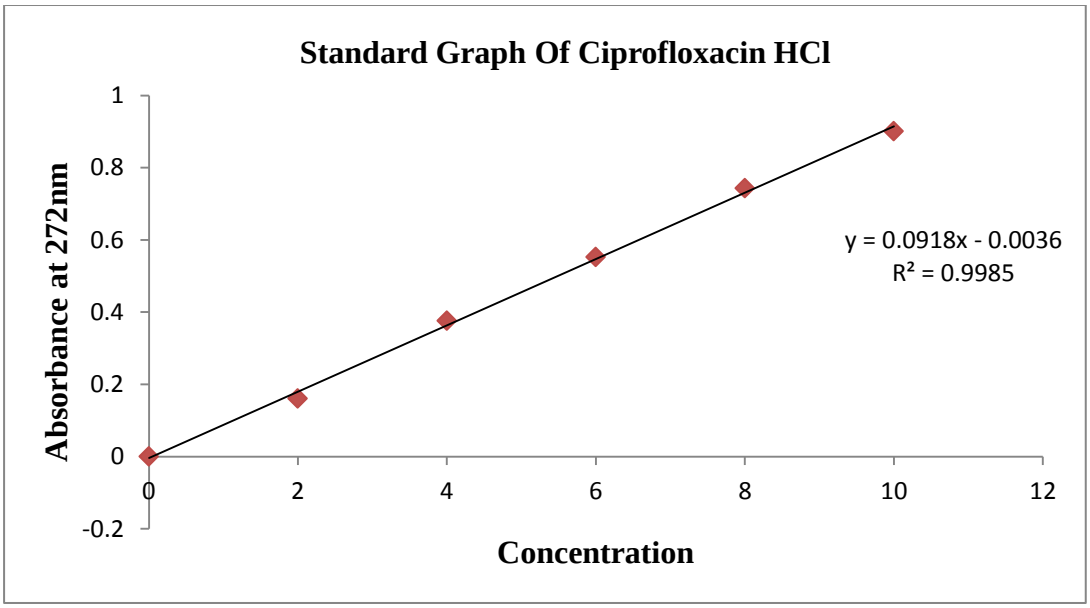


Fig 2:Calibration curve of an Ciprofloxacine HCl

From the standard curve of ciprofloxacin it was observed that the drug obeys Beer's law in the range of 2-20 µg/ml.

FT-IR Studies:

FT-IR Spectra of Drug: Ciprofloxacine HCl

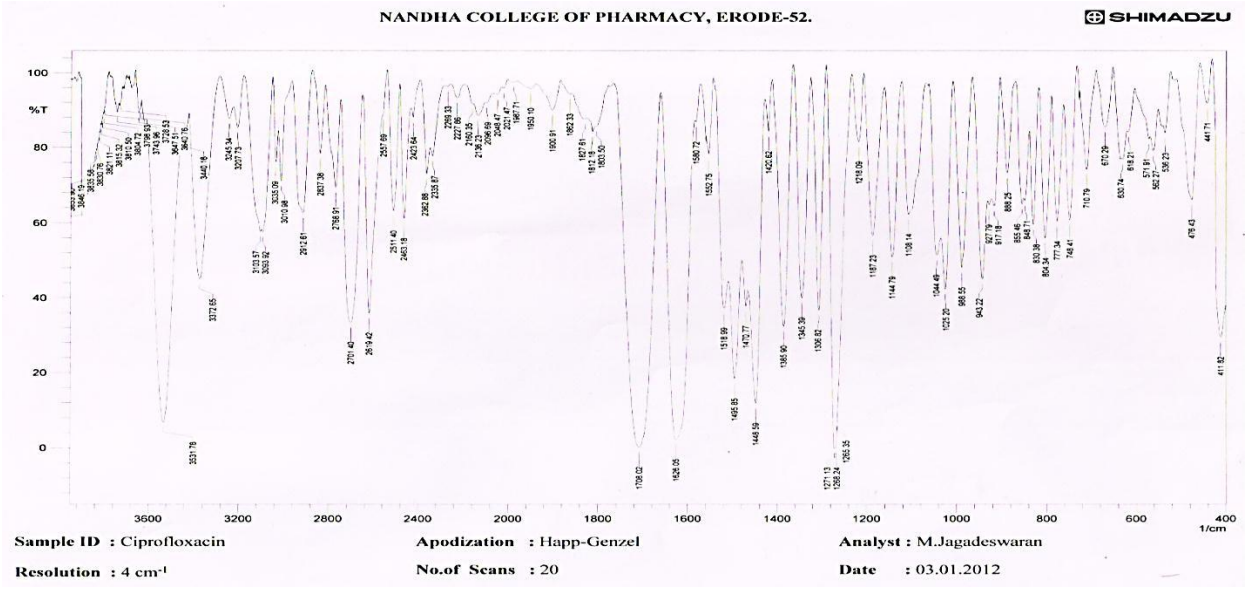


Fig 3: FTIR Spectroscopy of pure drug

Fig 4:FT-IR Spectra of Polymer: Sodium CMC

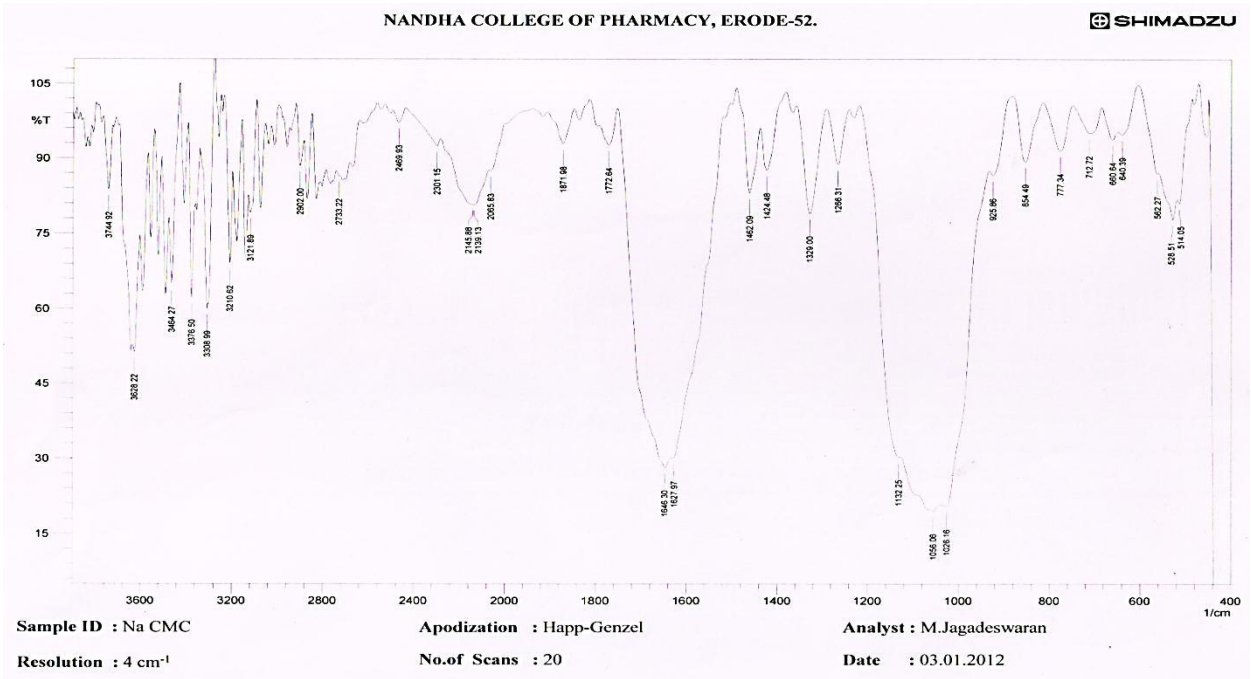


Fig 5:FT-IR Spectra of Polymer: Sodium Aliginate

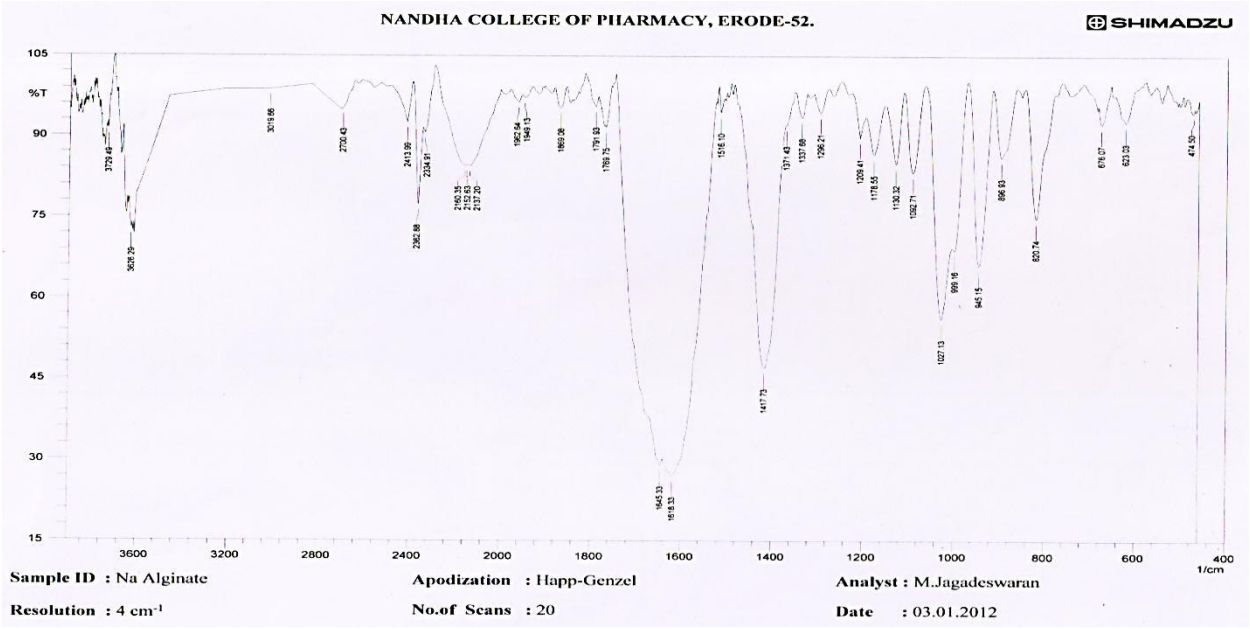


Fig 6:FT-IR Spectra of Polymer: HPMC

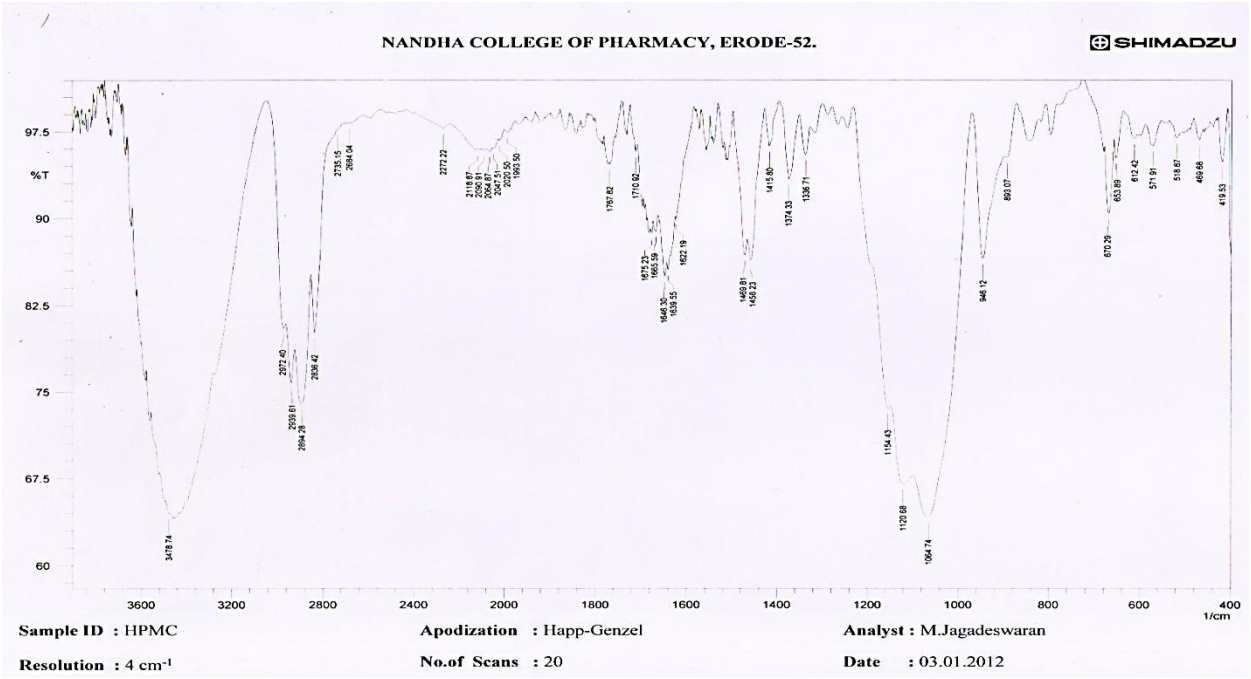


Fig 7:FT-IR Spectra of Drug with Sodium CMC:

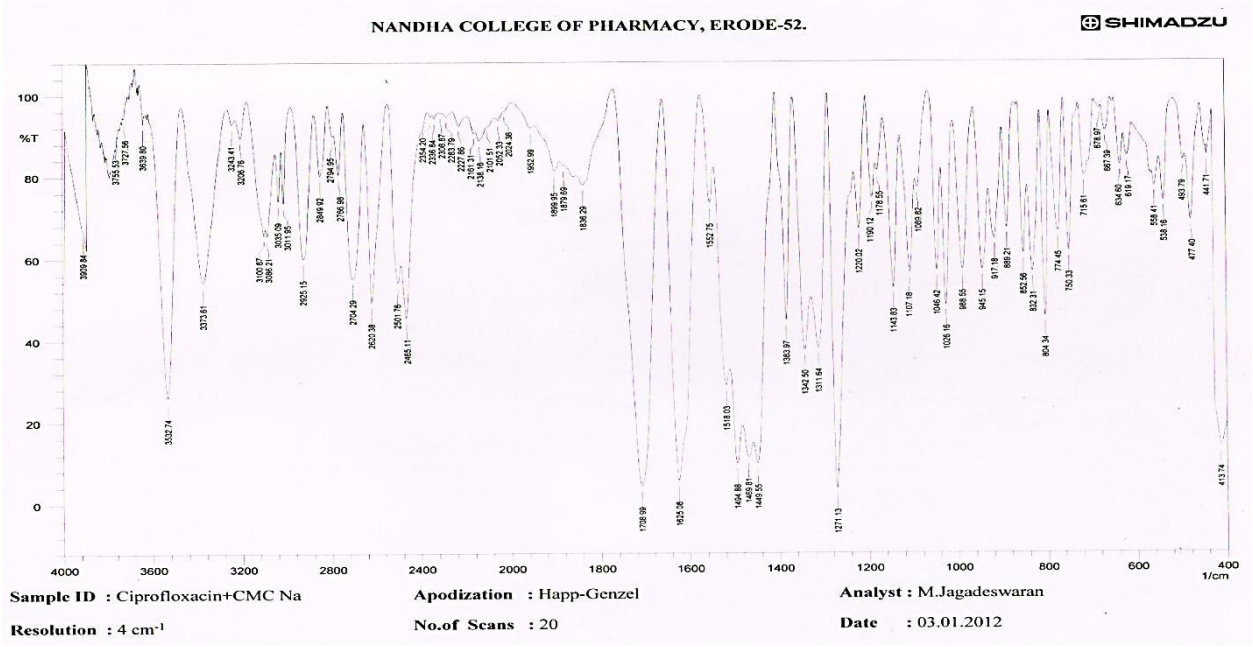


Fig 8:FT-IR Spectra of Drug with Sodium Aliginate

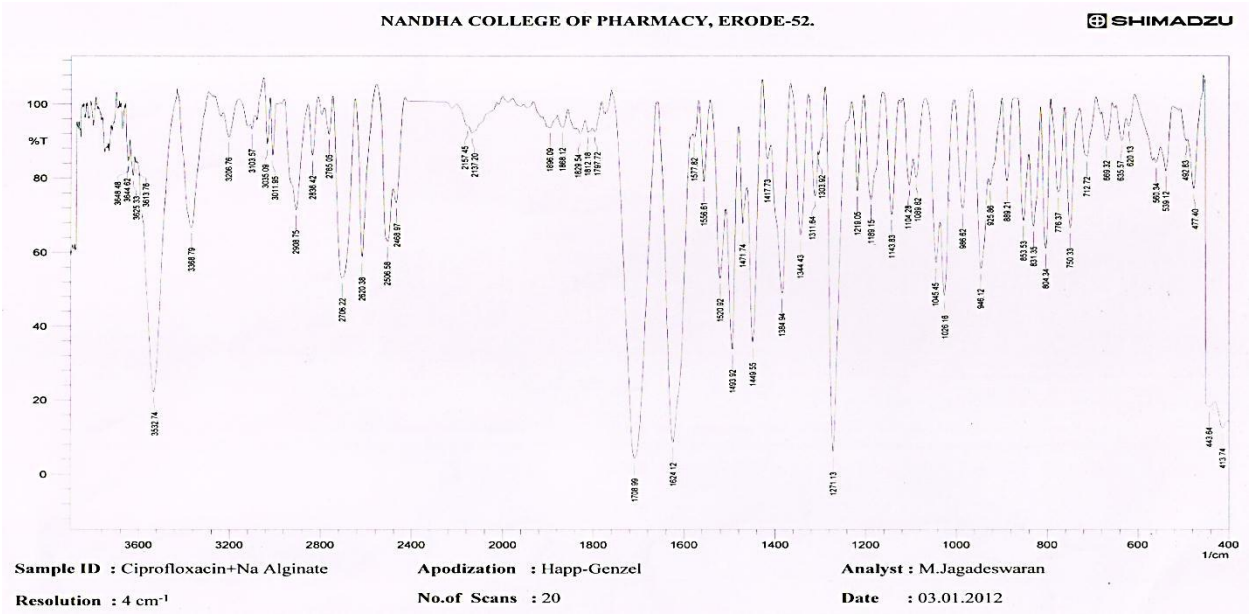


Fig 9:FT-IR Spectra of Drug with HPMC:

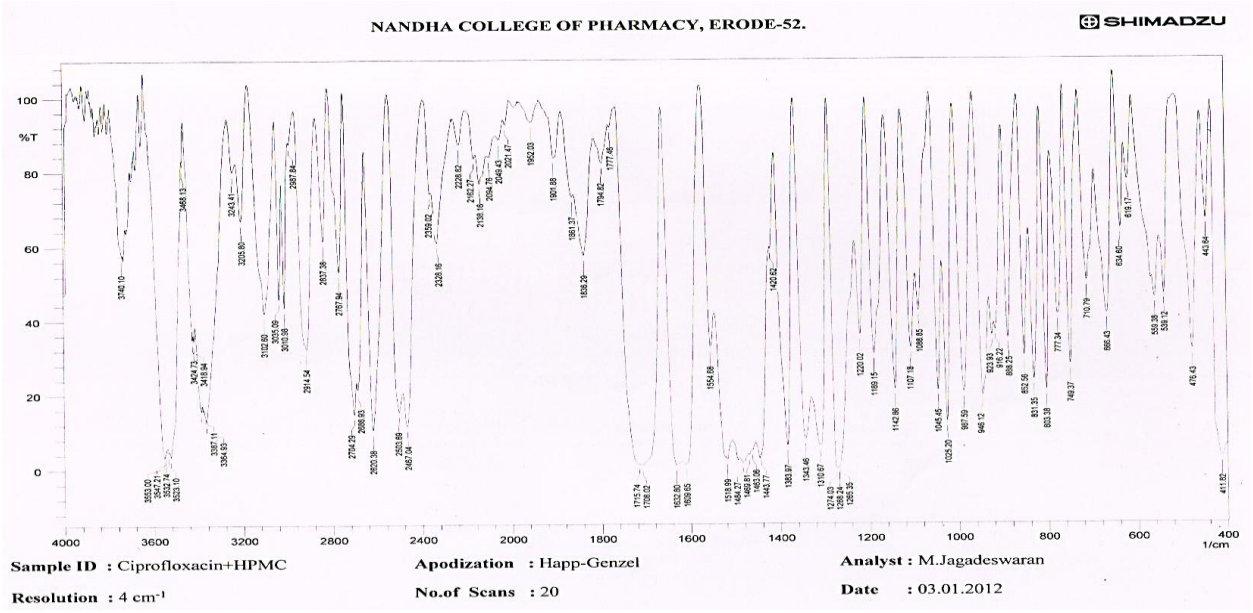


Table 7:

S. No	Type of bond	Standard wave number		Observed wave number		
			ciprofloxacin	CIP + Sodium CMC	CIP +Sodium Alginate	CIP +HPMC
1	C-H Alkanes (stretch)	3000 - 2850	3010.98	2902.00	3011.95	2839.38
2	-CH_3 Bend	1450	1448.85	---	1449.5	1443.77
3	-CH_2 Bend	1465	1385.90	1464.81	1471.09	1469.81
4	Aromatics	3150-3050	3035.09	3035.49	3036.21	3035.46
5	C=O	1725-1705	1708.02	1708.99	1624.12	1708.23
6	C-O	1300-1000	1306.02	1026.16	1028.16	1045.45
7	O-H	3650-3600	3624.31	3626.29	3624.33	3740.10
8	N-H	3500-3100	3531.78	3532.44	3532.74	3531.81
9	C-N	1350-1000	1345.39	1342.50	1344.43	1343.46
10	O-H	3400	3374.81	3376.30	3368.79	3364.93

Drug polymer interaction was checked by comparing the IR spectra of the formulations with the IR spectra of the pure drug. There was no significant change in the functional groups between the IR spectrum of the pure drug and also no additional peaks

were seen in the selected formulations. This confirms that no interaction between drug and excipients.

The drug-excipient interaction study was carried out using IR i.e. by KBr pellet method. In the drug-excipient interaction study, it was found that ciprofloxacin was having compatibility with all the excipients used in the formulation. It was found that all peaks corresponding to different functional groups of pure drug were present in the drug-excipient mixture, this shows the absence of interaction between the drug and excipients listed in above table.

Evaluation parameters:

Table 8: Particle size, Bulk Density, Drug content, Entrapment efficiency and mucoadhesive strenth:

Formulation	Particle Size (μm)	Bulk Density (gm/cm)	Drug content (%)	Entrapment Efficiency (%)	Muco Adhesive Strenth(%)
F1	97.21±0.57	0.271±0.21	82.56±0.34	72.72±0.13	64.11±015
F2	102.67±0.13	0.521±0.13	86.2±0.29	68.25±0.15	62.17±0.20
F3	74.75±0.12	0.47±0.17	83.15±0.88	67.71±0.21	58.14±0.19
F4	82.13±0.21	0.574±0.13	85.32±0.41	52.32±0.81	48.72±0.23
F5	68.83±0.32	0.596±0.12	76.51±0.27	58.21±0.21	46.26±0.72
F6	56.63±0.24	0.672±0.19	79.72±0.21	49.32±0.89	38.12±0.25

Partical size Analysis:

The particle size of the microspheres was determined by optical microscopy. The average particle size was found to be in the range of 61.4 to 199.9 μm. Batch F6 showed the least particle size of 56.63±0.24 μm which is due to spherical nature of microspheres as showed by the SEM. It was investigated that on increasing the concentration of polymer then particle size increases. The mean particle size of microspheres increased from 56.63±0.24 μm

to 102.67±0.13 μm with increase in concentration of polymer from 1 to 3%. The particle size of microspheres increased with the increase in the concentration of polymer, since at higher concentrations of the polymer solution dispersed into larger droplets, at concentrations lower than the optimum level the solution became less viscous and dispersed into various fine droplets that easily cosealed, resulting in larger microspheres.

Drug content:

The drug content estimation data for all the batches were found to be between 76.51 ± 1.27 to 86.2± 1.2 which was found to be within the limit and the result are by increasing polymer concentration, increasing the particle size.

Drug entrapment efficiency

Drug content in different formulations was estimated by UV Spectrophotometric method. Percent drug loading efficiency of microspheres was found in the range of 49.32±0.89 to 72.72±0.13. Formulation F1 containing Sodium CMC showed maximum percentage drug loading about 73 % whereas formulation F6 containing blend of HPMC showed minimum percentage of drug loading about 50% because these microspheres are small in size which results more loss of drug from surface during washing of microspheres. Rank order of % drug loading of various formulations was found to be as follows:

F1 > F2 > F3 > F5 > F4 > F6

Mucoadhesive Property:

The Mucoadhesive property of the microspheres was evaluated by in vitro adhesion testing methods called *in-vitro* wash off test. This test was done with the help of USP disintegration apparatus in which beaker contained 1.2 pH buffer solution. The numbers of microspheres adhering to the tissue were calculated after 30 min, 1 hr and hourly at 4 hr. After determination it was found that batch F1 showed highest percent 65% mucoadhesion than other batches.

In-vitro Drug Release Study:

Table 9:Cumulative percentage drug release of formulations:

Sl.No	Time	CIP+SodiumCMC (1:1) F1	CIP+SodiumCMC (1:2) F2	CIP+Aliginate (1:1) F3	CIP+Aliginate (1:2) F4	CIP+HPMC (1:1) F5	CIP+HPMC (1:2) F6
1	0	0	0	0	0	0	0
2	1	18.389±0.45	16.487±0.35	25.652±0.12	24.188±0.36	34.985±0.45	39.213±0.25
3	2	28.789±035	25.366±0.49	40.891±0.24	35.708±0.25	38.678±0.25	54.453±0.39
4	3	43.473±0.26	48.766±0.25	57.813±0.26	52.274±0.35	52.027±0.35	56.391±0.45
5	4	52.457±0.16	59.451±0.16	65.490±0.35	57.392±0.39	55.482±0.16	59.622±0.45
6	5	65.754±0.46	68.955±0.38	72.804±0.25	58.256±0.45	57.235±0.28	64.607±0.25
7	6	73.357±0.31	72.957±0.49	78.210±0.45	64.882±0.41	67.299±0.48	69.037±0.39
8	7	78.752±0.42	75.759±0.28	83.114±0.31	69.584±0.43	72.651±0.42	72.637±0.19
9	8	80.656±0.48	79.952±0.39	86.557±0.41	76.496±0.48	79.112±0.39	76.791±0.42
10	10	81.854±0.28	80.542±0.25	9.966±0.26	87.921±0.34	84.835±0.19	79.284±0.37
11	12	97.057±0.19	95.151±0.15	94.384±0.25	91.376±0.36	87.789±0.25	83.133±0.15

*Avarage readings of triplicate studies.

Fig 10:*In-vitro* drug release of microsphere:F1-F3

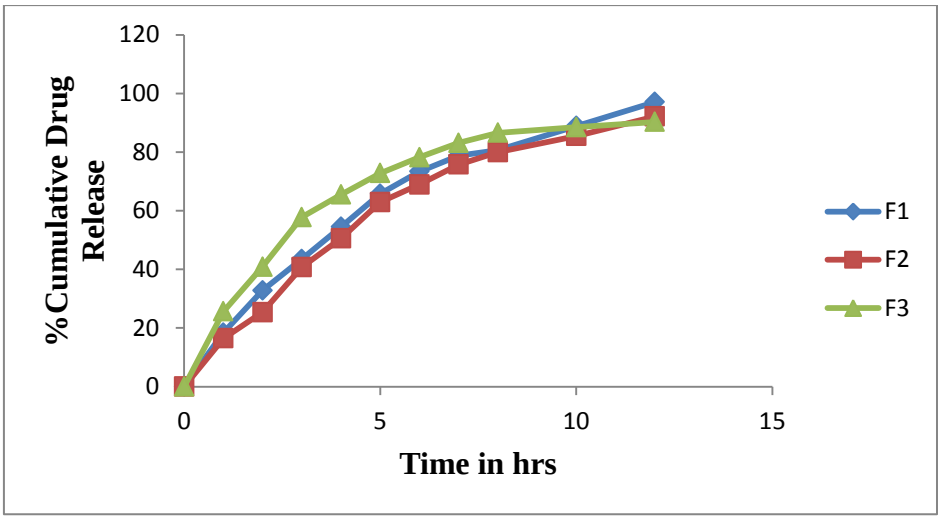
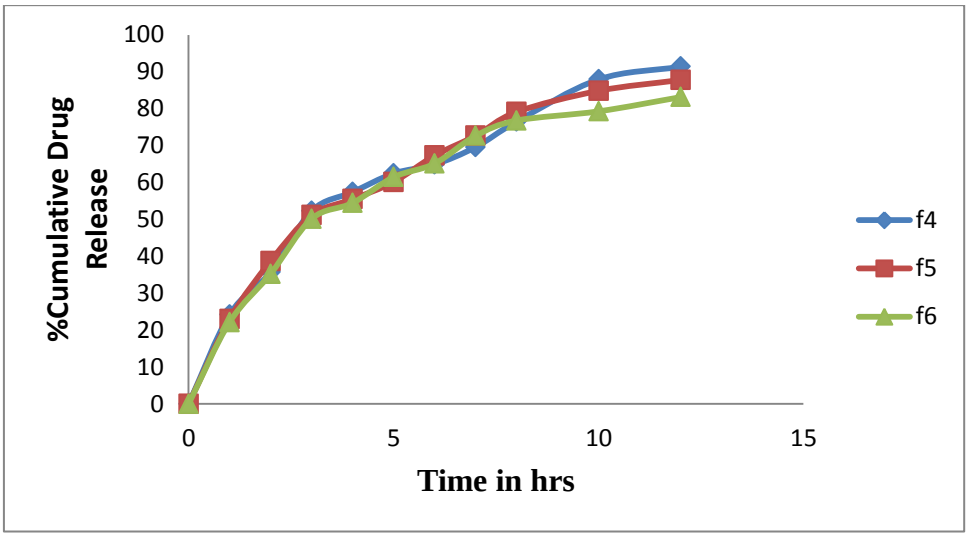


Fig 11 :*In-vitro* drug release of microsphere:F4-F6



In-vitro dissolution studies were performed for all the formulations using USPXXII Tablet dissolution tester employing paddle type at 500 rpm using 900 ml of 0.1N HCl as dissolution medium. The samples withdrawn were analyzed by using UV spectrophotometer. As per the results (Table.9) of dissolution study formulations F1, F2, F3, F4, F5 and F6 showed 97.05%, 96.15%, 94.38%, 91.37%, 87.78% and 83.13% respectively. This showed that the drug release from the caspule was sustained for 8 to 12 hr. shows reasonable drug release when compared to other formulations. Also all other parameters like drug content and particle size for these formulations were within the range. So, formulations F1 (Sodium CMC) were selected as the best formulation. The results are shown in table. 9.

Release kinetics:

Table 10: Model fitting for Optimized formulation F-1

Time in hrs.	Cumulative Drug Release (%)	Log of % Drug unreleased	Log t	SQRT	Log % Cumulative Drug Release
0	0	2	0	0	0
1	27.38	1.86	0	1	1.43
2	38.34	1.79	0.301	1.41	1.58
3	47.71	1.71	0.47	1.73	1.67
4	56.47	1.63	0.602	2	1.75
5	65.75	1.53	0.69	2.23	1.81
6	73.35	1.42	0.77	2.44	1.86
7	78.75	1.32	0.84	2.64	1.89
8	80.65	1.28	0.9	2.82	1.9
10	86.74	1.12	1	3.16	1.93
12	97.05	0.4	1.17	3.46	1.98

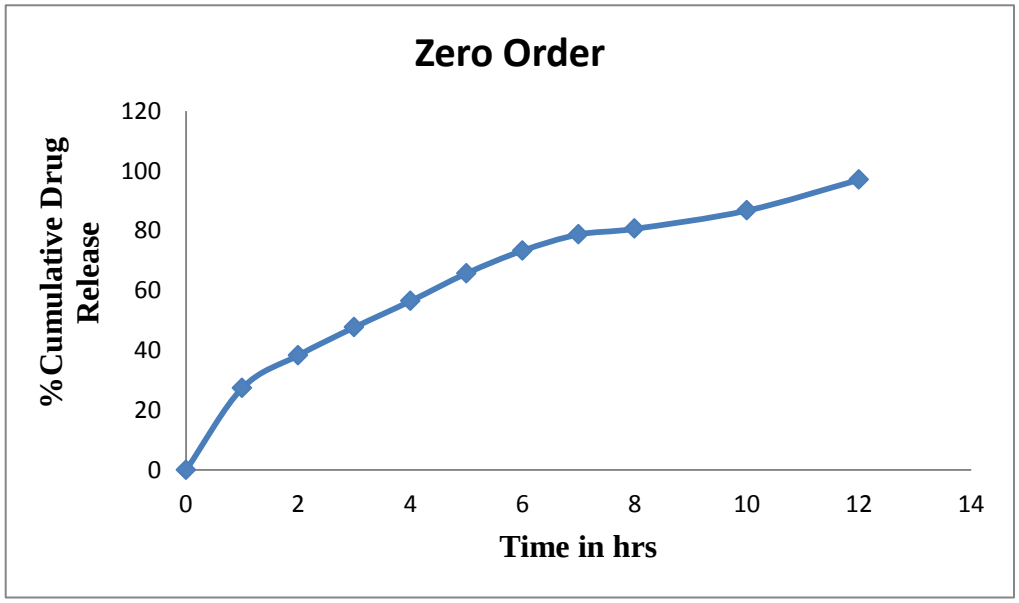


Fig 12: Zero order approximation for the formulation F1

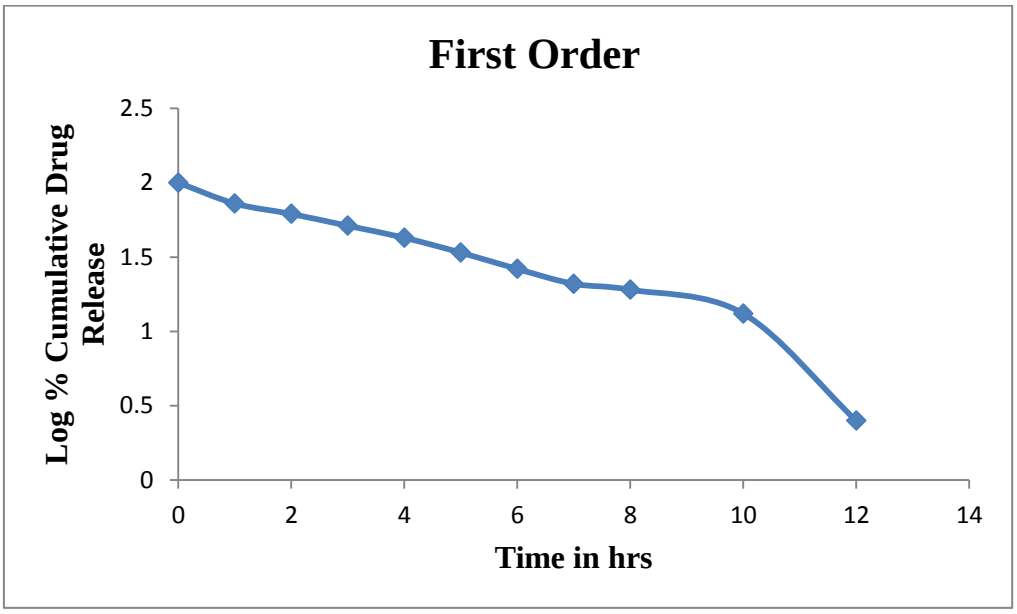


Fig 13: First order approximation for the formulation F1

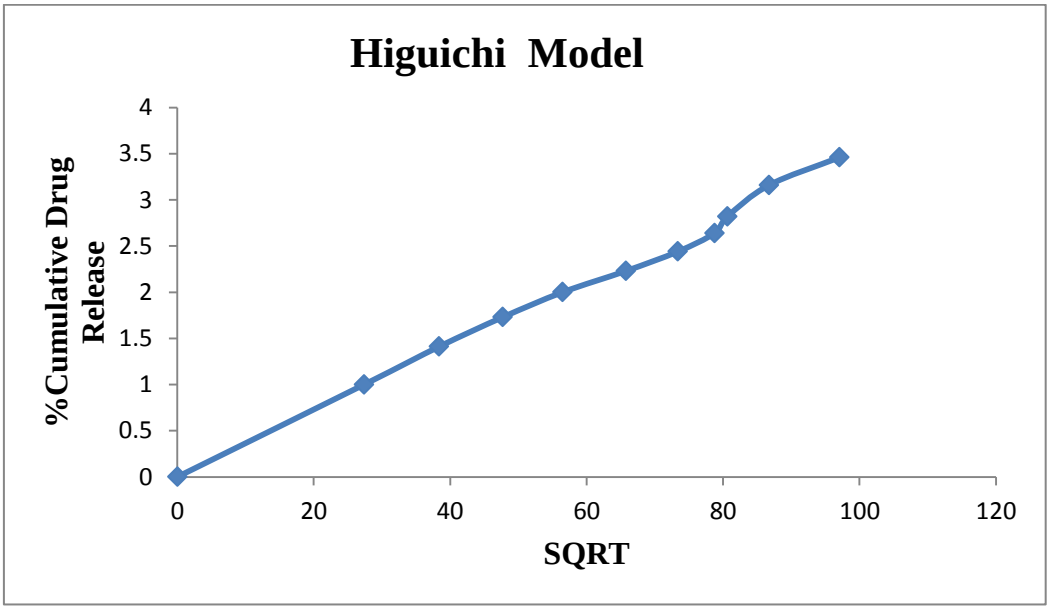


Fig 14: Higuchi's approximation for the formulations F1

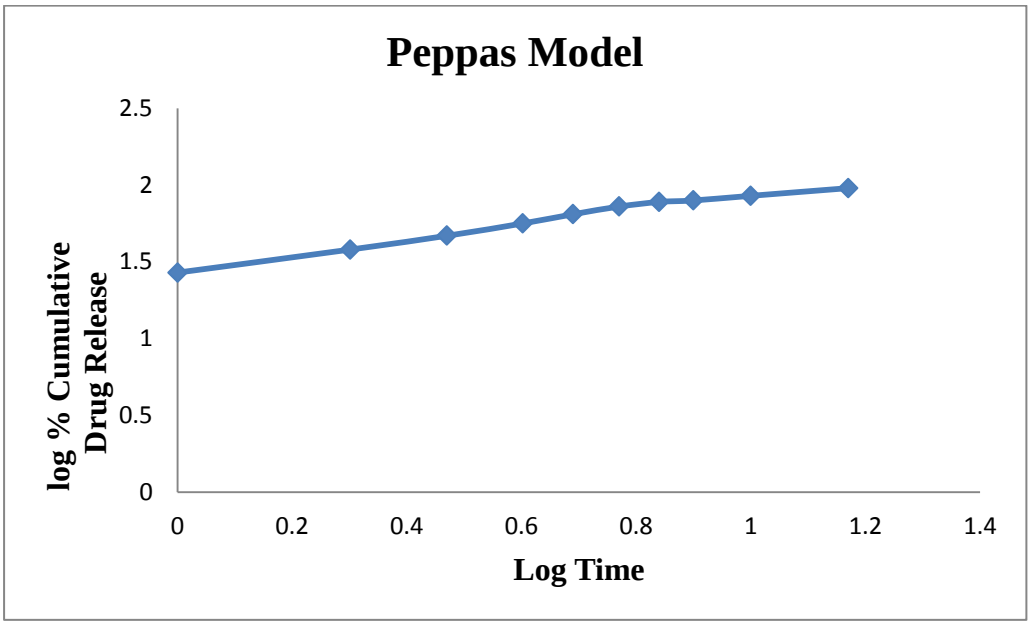


Fig 15: Korsmeyer-Peppas approximation for the formulations F1

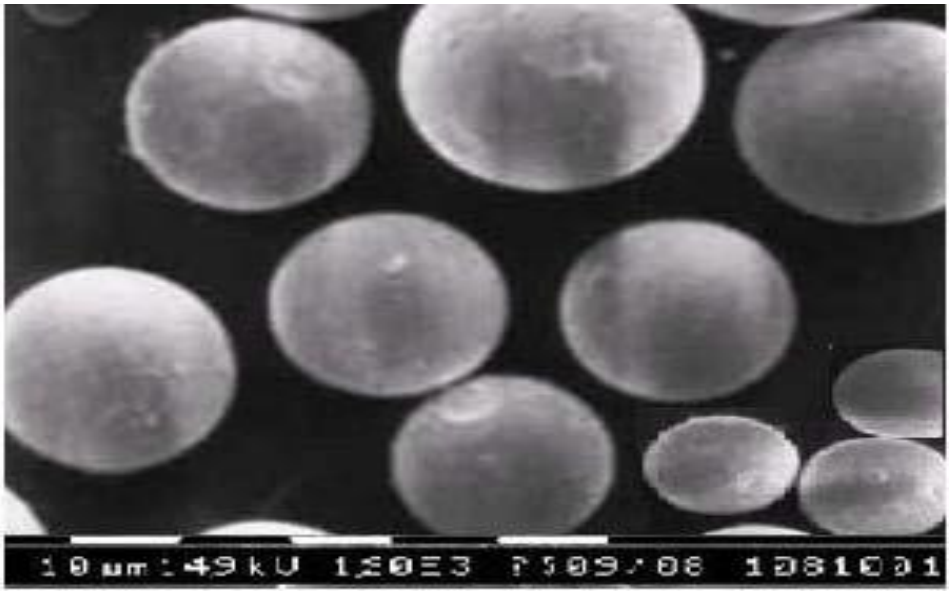
Table 11: Correlation coefficients of different mathematical models for formulations F-1

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Peppa's	
				R^2	n value
F1	0.894	0.916	0.993	0.986	0.49

The *in-vitro* drug release data of the floating tablets were evaluated kinetically by zero order kinetics; first order kinetics, Higuchi plot and Peppas models. The regression coefficient (R^2) value for Zero order, First order, Higuchi's, and Peppas plots for formulation F1 were found to be 0.894, 0.916, 0.993, 0.986 (Fig:12-15 and Table. 11) also plots were found to be linear, which indicates that the drug release depended on the square root of the time and predominantly controlled by diffusion process.

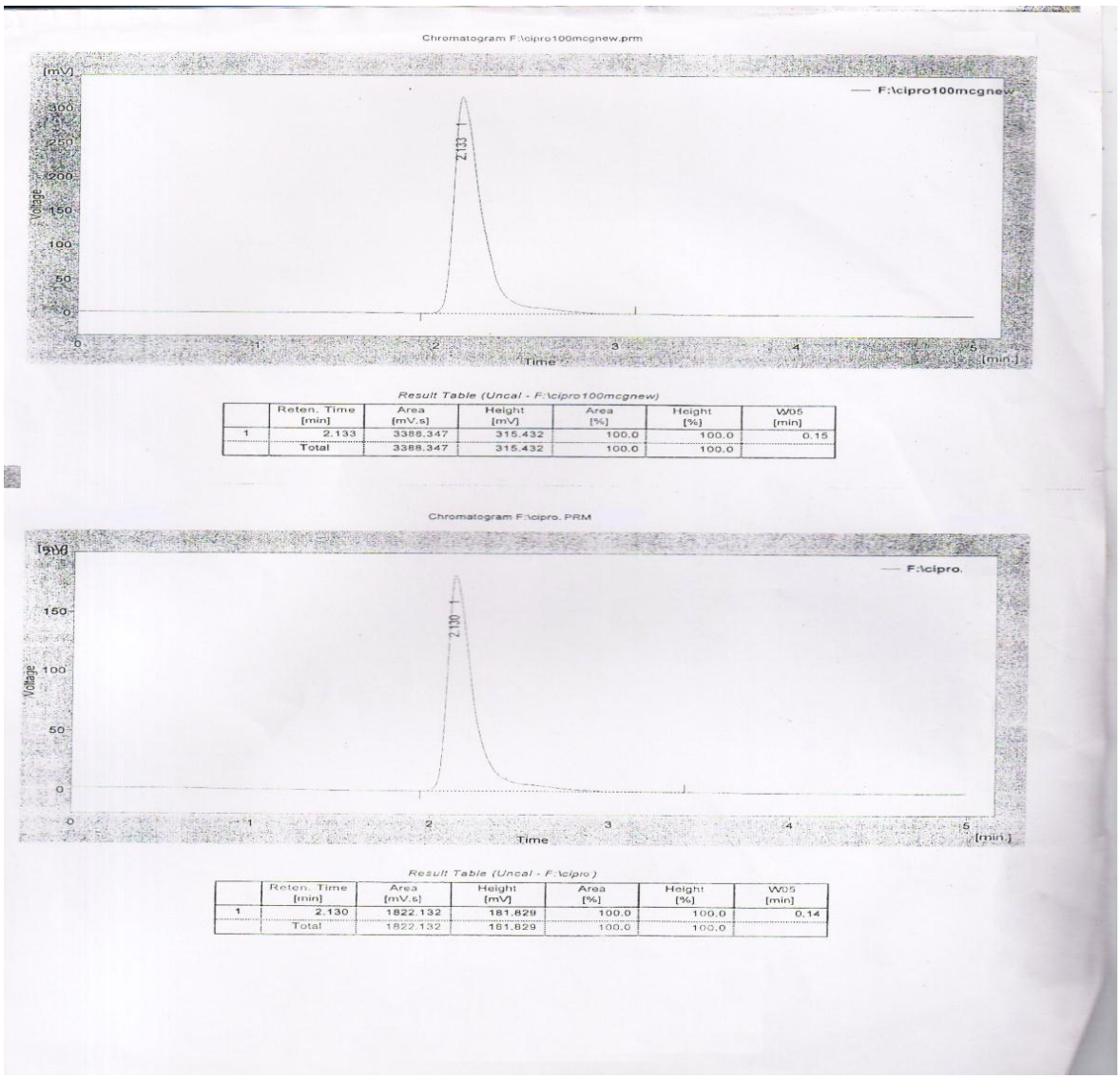
The mechanism of drug release is predicted by using Korsmeyer–Peppas equation. The n value of optimized formulation F1 was 0.49 and that of Fl formulation is less than 0.85. This indicates that the drug release depends on swelling, diffusion, and erosion. All formulations follow the non-Fickian or anomalous type of diffusion.

Fig 16: Particle size analysis and Surface Morphology of Microparticles by SEM:



Scanning Elrctron Microscopy of the prepared Ciprofloxacin HCl microspheres to access their surface and morphological characteristics as shown [Figure 16].

Fig 17: HPLC Analysis Of Formulation F1 with Marketed Drug (Cipro):



The Ciprofloxacin HCl content was determined by HPLC, and the experimental measurement was compared to the standard (Marketed) drug. The retention time for Ciprofloxacin (standard) was found to be 2.133 min and the sample (F1) was found to be 2.130 min (fig no.17).

Stability Study:

Table 11: Stability study data for F1

Sr. No.	Days	% R.D.C. 5-8^{O}C	% R.D.C. 27 ± 2^{O}C	% R.D.C. 40 ± 2^{O}C
1	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
2	15	99.97 ± 0.024	99.95 ± 0.018	99.94 ± 0.034
3	30	99.84 ± 0.032	99.81 ± 0.026	99.73 ± 0.041
4	45	99.63 ± 0.045	99.54 ± 0.031	98.44 ± 0.037
5	60	99.24 ± 0.038	98.93 ± 0.021	98.80 ± 0.026

*Average of three readings

R.D.C. = Remaining Drug Content # = R.D.C. In pH 1.2 (0.1N HCl)

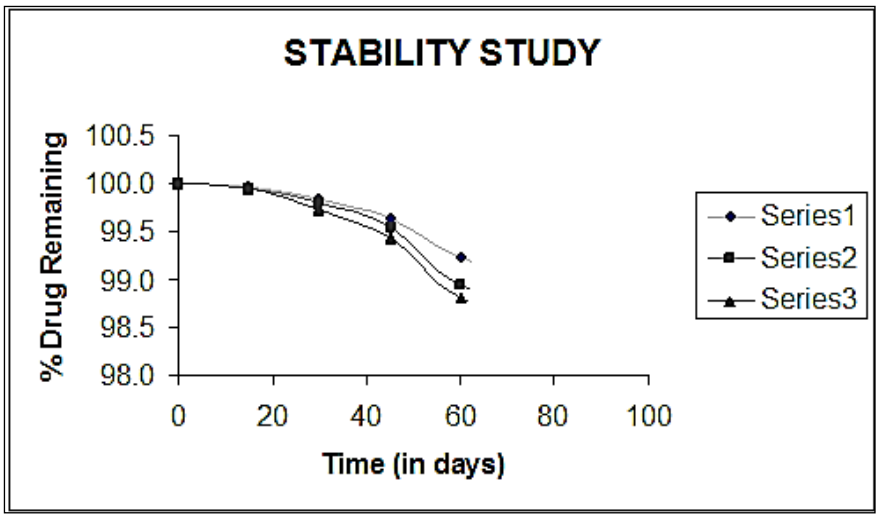


Fig 18: Stability study

Stability study is the important part of the study for any pharmaceutical formulation. There are procedures given for the stability study in ICH guidelines. In response to that stability study was carried out for the formulation F1(1:1) by exposing it to temperature 5-8^{o} C, 27^{o} C and 40^{0} C for 2 months. The sample was analyzed for drug content at regular interval of two months and it was evident that there was no remarkable change in the drug content of

microparticles. Results show that formulation F1 was stable at mentioned temperatures. As the drug used, Ciprofloxacin HCl, is recommended to be stored below 25^0 C, stability study has not been performed above ambient temperature.

SUMMARY

Sustained release drug delivery system are retained in the mucus membrane of GI for a longer time and assist in improving the sustained release of drugs that have an absorption window in the particular region of the GI tract as well as for controlling the release of the drug having site-specific absorption limitation.

Ciprofloxacin HCl is a fluoroquinolone antibacterial agent which is highly effective against gram positive and gram negative bacteria, was used as a model drug to develop a sustained release formulation. Ciprofloxacin HCl exhibits pH dependent solubility. It is more soluble in acidic pH and slightly soluble at neutral or alkaline condition (intestinal environment). Hence an attempt was made to develop gastroretentive delivery system of ciprofloxacin which increase the bioavailability of ciprofloxacin and also to reduce frequency of administration, thereby improving patient compliance and therapeutic efficacy.

In the present work, 6 different formulations were prepared by emulsificaion solvent evaporation method using three different polymers. They are Sodium Corboxy Methyl Cellulose(1:1 and 1:2), Sodium Alginate(1:1 and 1:2) and HPMC K100M (1:1 and 1:2).

Characterization of the drug was done by performing the UV spectroscopy and IR spectroscopy. IR spectrum of the pure drug was compared with that of physical mixture of drug with all the excipients used in the study. The results showed that there was no drug-excipient interaction.

All the prepared formulations were evaluated for Bulk density, Entrapment efficiency, drug content uniformity, Mucoadhesive strength, drug-polymer interaction, *in-vitro* drug release, Scanning electron microscopy, HPLC and short term stability studies.

The dissolution studies were carried out for 12 hrs. As per the result of dissolution study formulations(Table:9) F1(1:1), F2(1:2), F3(1:1) and F4(1:2) showed reasonable release 97.05, 95.151, 94.38 and 91.37 %, respectively. F1 and F2 showed good drug release profile, when compare to other formulations. Based on all these results, formulations F1 was selected as the best formulation.

The release kinetics were fitted to different mathematical models like Zero order, First order, Higuchi's and Peppas plot. The selected formulations F1(1:1) follows Higuchi's plot and slope (*n*) value of Peppas for these formulations were found to be in the range less than 0.85. This indicates that the drug release depends on swelling, erosion, and diffusion.

These formulations follow the non-fickian or anomalous type of diffusion. The drug-polymer ratios, viscosity of Sodium CMC, were found to influence the drug release.

The drug polymer interactions of the optimized formulations were evaluated by FTIR. FTIR spectrum of pure drug was compared with that of formulations F1, F2, F3, F4, F5 and F6. All peaks corresponding to the different functional groups of pure drug were present in the formulations which indicate the absence of interaction between the drug and excipients(Table.7).

The selected formulation F1 (1:1) was subjected for stability studies as per ICH guidelines. Formulations subjected for short term stability studies were checked for drug content, entapment efficiency, Mucoadhesive strength and physical appearance for 90 days with an interval of 15 days. The formulations were found to be stable as no significant change was observed in the various evaluated parameters like HPLC of the formulation.

CONCLUSION

From past few years microparticles have been studied by many workers as a choice of sustained drug delivery system to provide a better drug bioavailability considering, high penetration property of the microparticles encapsulated agents through biological membrane and the stability of them.

The present formulation study on Ciprofloxacin HCl is an attempt to prepare microparticle drug delivery system and evaluate its performance, *In-vitro.* The formulations were prepared, varying the ratios of polymer by emulsification solvent evaporation method.

An ideal or best formulation of microparticles is the one which gives high entrapment efficiency along with good stability, retention time (by using HPLC) and drug release profile. In the present study entrapment efficiency is found to be drug and polymer ratio dependent. The release rate is found to be depended on polymer concentration.

In the present study entrapment efficiency is found to be dependent on drug and polymer ratio. The formulation F1 (1:1), which showed higher entrapment efficiency and muco adhesive strength provides desired drug release rate.

By these facts, study can be concluded by saying that Ciprofloxacin among the Mucoadhesive microspheres of Ciprofloxacin HCl prepared using Sodium CMC, Sodium Alginate, HPMC polymers using concentrations of 1:1 and 1:2 respectively, is a promising approach to enhancing the bioavailability of Ciprofloxacin HCl. The formulations F1, F2 showed reproducible results and the best Mucoadhesive profile with good surface morphology. The method employed gave spherical, discrete, and free flowing microspheres of Ciprofloxacin. Among all the formulations of microspheres, Sodium CMC with 1:1 praportions showed the best sustained release mucoadhesive effect.

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